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Blueprint Neurotherapeutics Network (BPN)—Small Molecules

Poster #20

Thota Ganesh, Ray Dingledine, Nicholas Varvel, Radhika Amaradhi, Avijit Banik, Wenyi Wang
EP2 Antagonism for mitigating cognitive and memory consequences following status epilepticus

Prostaglandin-E2 receptor EP2 is a Gαs-protein coupled receptor, upon activation by endogenous PGE2, induces cAMP via adenylyl cyclase enzyme. Induction of cAMP is coupled to protein kinase-A (PKA), or exchange protein activated (Epac)-mediated downstream mechanisms, functionally leading to either neuroplasticity or neuroinflammation in acute brain injury conditions such as traumatic brain injury, seizures, status epilepticus, multiple sclerosis, and also in the chronic neurodegenerative diseases such as epilepsy, Alzheimer's disease, and Parkinson's disease. We have created several small molecule antagonists for EP2 beginning from high-throughput screening, structure activity relationship studies, and lead optimization approaches. We also tested selected best agents to study the functional implications of EP2 receptor in status epilepticus (> 30 min of continuous seizures), Alzheimer's disease and stroke, glioblastoma models. In parallel, Pfizer, and Amgen has created and tested their molecule in *in vitro* and *in vivo* models. All the results suggest EP2 is a novel target for drug discovery. We have recently demonstrated that exposure to a brief period of EP2 antagonist in mice after 1 hour of status epilepticus, has several beneficial effects including attenuation of mortality, neuroinflammation, and neurodegeneration. Moreover, EP2 antagonist treatment repairs the compromised (leaky) blood-brain barrier and protects from memory impairments in mice after status epilepticus. Furthermore, we recently advanced a candidate EP2 antagonist for preclinical development for the treatment of cognitive and memory deficits following status epilepticus. We will present a mechanistic and functional rationale for targeting the EP2 receptor, its strengths, and potential weaknesses; opportunities and threats targeting the EP2 receptor, along with medicinal chemistry lead-optimization efforts, proof-of-concept/efficacy studies from pharmacological approaches *in vitro* and *in vivo* animal models.

Poster #16

Jay P. McLaughlin, Shainnel O. Eans, Bowen Tsai, Ariana C. Brice-Tutt, Dmitry Yakovlev, Brian I. Knapp, Jean M. Bidlack, Jane V. Aldrich
Probing analogs of macrocyclic tetrapeptide CJ-15,208 for orally-active kappa opioid receptor antagonism as a potential therapeutic treatment to prevent stress-induced cocaine reinstatement

The macrocyclic tetrapeptide natural product *cyclo*[Phe-D-Pro-Phe-Trp] (CJ-15,208) and its stereoisomer *cyclo*[Phe-D-Pro-Phe-D-Trp] ([D-Trp]CJ-15,208) both demonstrate kappa opioid receptor (KOR) antagonist activity following oral administration, preventing stress-induced reinstatement of cocaine-seeking behavior. To further explore the structure-activity relationships, enhance KOR antagonist activity and expand the potential therapeutic applications of KOR antagonism for the treatment of cocaine use disorder, we examined 48 macrocyclic tetrapeptide analogs in vitro with competition binding assays using the KOR-selective radioligand [^{3}H]U69,593, identifying 26 that possessed KOR affinity with a K_i value of 50 nM or less. Of these, analog BPN-37088 demonstrated a KOR K_i value of 3.2±0.5 nM, less than half that of [D-Trp]CJ-15,208, and antagonized KOR-agonist-inhibition of stimulated cAMP production in hKOR-CHO cells with an IC_{50} value of 23±7.7 nM. Screening results were confirmed *in vivo* with mice administered analogs orally (at 30 mg/kg, p.o.) and tested for their ability to antagonize the antinociception of the KOR-selective agonist U50,488 (10 mg/kg, i.p.) in the 55°C warm-water tail-withdrawal test. Consistent with the *in vitro* screening, a number of analogs produced KOR antagonism after a 2.5 h pretreatment. Upon further characterization, BPN-37088 produced dose-dependent and selective antagonism lasting at least 2.5 h after U50,488 was administered either

peripherally (10 mg/kg, i.p.) or centrally (100 nmol, i.c.v.) with doses as low as 1 mg/kg, p.o.. BPN-37088 was over ten-fold more potent than the parent peptide [D-Trp]CJ-15,208. In further oral testing, pretreatment with BPN-37088 (3 mg/kg, p.o.) prevented stress-induced reinstatement of extinguished cocaine conditioned place preference. Collectively, these data demonstrate the progress of the current project to enhance KOR antagonist activity of the analogs for potential development, as well as the therapeutic potential of KOR antagonists to prevent relapse to drug-seeking behavior in abstinent subjects. (Supported by UG3 NS132600-01 from NIH).

Poster #15

Mehrdad Shamloo

Differential modulation of cannabinoid receptor 2 signaling, internalization, and desensitization by biased agonism for treatment of methamphetamine addiction

America currently faces a dire epidemic of substance use disorders. Stimulant abuse significantly contributes to this social crisis, with 1.6 million suffering from methamphetamine (METH) use disorder and another 1.4 million from cocaine use disorder. METH and cocaine are the major causes of overdose deaths behind opioids. Methadone and buprenorphine are widely used treatments for opioid addiction, but there is currently no approved pharmacological treatment for stimulant use disorder (SUD). Thus, there is an urgent need to develop pharmacological interventions for SUD. The cannabinoid receptors 1 and 2 (CB1R and CB2R) are part of the endocannabinoid system and play a crucial role in modulating dopamine (DA) levels in the central nervous system. The CB2 receptors of dopaminergic neurons in the ventral tegmental area play an inhibitory role in this dopaminergic circuit, leading to reduced DA release in the nucleus accumbens. Thus, selective modulation of CB2R may lead to the development of new therapeutic interventions for substance use disorders. One challenge for treatment development targeting CB2R is that this receptor engages two cellular signaling cascades: the cAMP and beta-arrestin pathways. Differential activation of these signaling cascades leads to receptor desensitization/internalization. Receptor desensitization have been the cause of the failure of unbiased CB2R agonists in past clinical trials for pain; these medications temporarily relieved pain but quickly lost their efficacy. With support from NIDA and the Blueprint Neurotherapeutics (BPN) program, we successfully identified a lead series of novel chemical entities (NCEs) that display functional selectivity (i.e., bias) for cAMP over arrestin and are also very selective for CB2R over CB1R. In addition, our NCEs have demonstrated efficacy in METH addiction animal models. This class of compounds is now being optimized for their drug-like properties for potential clinical development for METH use disorder.

Poster #13

A. J. Shepherd, C. M. Gaffney, B. R. Varga, L. Gonzalez, K. M. Valadez, N. T. Nguyen, L. Araldi, s. Duddy, P. Pearson, S. Young, M. Surman, S. Majumdar, V. Cherezov, V. Katritch

Non-addictive Angiotensin AT2 Inhibitors for Neuropathic Pain Relief

Neuropathic pain affects more than 20 million Americans and costs the economy more than $560 billion per year. Long-term use of opioids to manage such pain is associated with side-effects, addiction and overdosing. Other first-line treatments such as anti-depressants and anticonvulsants have sub-optimal efficacy; it is estimated that fewer than one in four patients with neuropathic pain experience 50% or greater pain relief with current treatment options.

Antagonists of the angiotensin AT2 receptor (including EMA401 and CFTX-1554) have been developed for neuropathic pain relief and have shown efficacy in multiple neuropathic pain models and clinical trials. However, studies with EMA401 were terminated prematurely due to hepatotoxicity concerns.

Therefore, our goal was to develop and characterize AT2R antagonists for neuropathic pain indications that circumvent the hepatotoxicity concerns encountered by EMA401. We used SAR guided by recent solving of the AT2 receptor crystal structure to guide development of a series of iterations on initial hits from a compound library. Hits were screened *in vitro* and those that also exhibited favorable ADME properties were tested in the mouse spared nerve injury model of neuropathic pain.

Our team has identified a lead candidate molecule with excellent efficacy, ADME properties and favorable pharmacokinetics in mice, rats and dogs. We have shown 80% reversal of pain hypersensitivity for >8 hours with oral dosing in the mouse SNI model of neuropathic pain at 10mg/kg. The estimated human dose would be 50 mg daily, with no drug-drug interaction liabilities. Future IND-enabling studies are currently underway.

Poster #14

M. Surman, C. Alves Jesus, W. Childress, P. Harrington, R. Lewis, K.W. Fowler, J. Patel, K. Guenther, C. D. Peterson, K. F. Kitto, R.B. Franklin, W.H. Martin, C.A. Fairbanks, A.G. Hohmann, S.K. Florio

Discovery of PSD95 protein-protein interaction inhibitors as novel non-opioid analgesics

Pain is responsible for more encounters with the health care system than any other single cause, yet treatment options for neuropathic pain have limited efficacy and carry a high risk for side effects, including opioid addiction. Glutamate activation of N-methyl-D-aspartate (NMDA) receptors mediates central nervous system (CNS) sensitization, which is implicated in the development and maintenance of neuropathic pain. NMDA-mediated central sensitization depends on formation of a multi-protein complex at the receptor consisting of the NMDA receptor, the scaffolding protein, postsynaptic density protein 95 (PSD95), and neuronal nitric oxide synthase (nNOS). By bringing these proteins close together, multiple signaling cascades are activated leading to neural network reorganization (plasticity). Small molecules and cell penetrating peptides that disrupt this complex act as effective analgesics in preclinical animal models with better side effect profiles than non-selective NMDA receptor antagonists and nNOS inhibitors.

Anagin, a preclinical stage company, in partnership with the BPN for small molecules program (https://neuroscienceblueprint.nih.gov/neurotherapeutics/bpn-small-molecules) and Anagin's collaborators, has advanced compounds into the lead optimization phase of small molecule drug development. *In vitro* and *in vivo* studies have confirmed target engagement by the compounds and suggested a tissue-based potency of 1 to 100 nM. Compounds in our lead series are well absorbed, well-tolerated and brain penetrant. *In vitro* profiling studies suggest a promising safety margin for our series. Our most advanced lead molecule is orally efficacious in rodent pain models with an effect size similar to 100 mg/kg gabapentin, *p.o.* (≤10 mg/kg, *p.o.* when administered >14 days post-surgery). Our goal is to identify one molecule as a clinical candidate in Q2, 2024 for advancement into IND-enabling studies (2024/2025). If successful, at the conclusion of these studies we will have a new clinical candidate thoroughly interrogated and poised for testing in clinical trials for chronic pain as the first orally available small molecule targeting PSD95-nNOS.

Blueprint Neurotherapeutics Network (BPN)—Biologics

Poster #2

Steve O'Connor
IND enabling non-clinical development of E1v1.11, a morpholino anti-sense oligonucleotide for the treatment of spinal muscular atrophy

The objective of this project is to develop a safe, effective antisense oligonucleotide (ASO) with Phosphorodiamidate Morpholino Oligomer (PMO) backbone chemistry capable of significantly reducing PMP22 protein production in Schwann cells, which is titratable in its dosing characteristics. For patients with CMT1A, the most common form of CMT, PMP22 protein over production drives disease progression. We have previously performed rigorous analysis of the *PMP22* gene sequence, which has led to the design of numerous ASOs that have been shown to effectively alter PMP22 mRNA production. These PMOs have been designed to alter exon splicing, resulting in an "exon-skipped" product that is severely truncated early in the translation process, effectively reducing PMP22 protein production. Importantly, however, PMP22 production is not completely eliminated since this would also be detrimental. CMT1A is a remarkably common rare disease that is caused by the overexpression of a protein involved in the function of peripheral nerves. The protein, PMP22, is a component of the myelin sheath which acts much like an insulator, allowing nerves to transmit their signal properly. While too much PMP22 is detrimental, too little of PMP22 is also problematic. Therefore, any therapeutic strategy cannot simply knock-out expression of this important gene. The molecular genetics of CMT1A makes this disease particularly amenable for nucleic acid-based therapeutics designed to modulate PMP22 expression since 1) the disease gene has been identified; 2) CMT1A is monogenic; and 3) diminution of PMP22 expression can be accomplished through a variety of molecular mechanisms. Currently, no CMT1A drugs are available, thus this highly at-risk patient class lacks any disease altering therapy and relies solely upon palliative care.

Previously, we have performed *in vitro* cellular assays to screen numerous PMO molecules against PMP22 mRNA. Select compounds were optimized and studied in C3 mouse models (containing multiple copies of the human *PMP22* gene) for efficacy and initial safety. We have identified a lead candidate (SHC1A-012) that has demonstrated both molecular and clinical outcome efficacy in a sustained manner. The efficacious dosing levels have proven safe with multiple injection doses and demonstrate broad biodistribution, as well as long effective duration even after administration was stopped for months. Initial studies indicate the drug will be deliverable (in humans) with a straightforward subcutaneous injection performed 2-4 times per year, providing a relatively straightforward treatment regimen to improve these patients' health. For this project, we will be expanding our efficacy studies in genetically engineered mice, studying safety and toxicity in both C3 mouse models as well as WT rats, and performing full biodistribution analysis in animal models. Finally, we will perform long-term efficacy studies to explore the trade-offs between phenotypic improvement and side effects of various SHC1A-012 dosing schemes.

HEAL Pain Therapeutics Development Program (PTDP)

Poster #34

Jianguo Cheng, Qingyuan Fan, Jihye Kim, Jie Zhang

Enhancing fractalkine signaling normalizes CCI-induced differential expressed genes and mitigates neuropathic pain

Objective: Neuropathic pain (NP) is one of the most prevalent and incapacitating forms of chronic pain. A better understanding of its pathogenesis at cellular and molecular levels is crucial to the development of new therapeutics. A major focus has been on the roles of Fractalkine (CX3CL1) signaling between neurons and immune cells in the pathogenesis of NP. In this study, we over-expressed CX3CL1 in mice and investigated the impact of over-expressing CX3CL1 on neuropathic pain and nerve injury-induced differentially expressed genes (DEGs). **Methods:** Chronic constriction injury (CCI) was performed on wild-type (WT) and CX3CL1 overexpressing (CX3CL1-Tg/CX3CR1GFP/+) mice. Mechanical hyperalgesia was evaluated using the von Frey Filament test. CCI-induced DEGs in the injured sciatic nerve in WT and CX3CL1-Tg mice in RNA sequencing were analyzed at post-CCI day 28. **Results**: Compared to WT mice, mice with full-length CX3CL1 overexpression showed significantly reduced pain behavior after CCI, as reflected by dramatically elevated paw withdrawal thresholds in the von Frey test in both male and female animals. We identified 5,882 CCI-induced DEGs (FDR<0.05 and a threshold of |log2 FC| > 1) in WT mice (CCI vs. Control). Of these genes, 3,224 (52.5%) were upregulated and 2,658 (47.5 %) were downregulated. The upregulated genes showed significant enrichment in multiple immune-related biological processes including cytokine/chemokine-mediated signaling and immune cell activation in Gene ontology (GO) enrichment analysis. CX3CL1 over-expression significantly normalized CCI-induced DEGs and signaling of relevant pathways. We identified 3,181 DEGs (FDR<0.05 and a threshold of $|\log_2 FC| > 1$) by comparing data from WT and CX3CL1-Tg mice; 1,442 (43.7%) were upregulated and 1,706 (56.3%) were downregulated after CCI. CX3CL1 over-expression almost fully reversed the upregulated genes in immune-related biological processes and partially reversed the downregulated genes related to synaptic transmission, synaptic organization, axonal outgrowth, and neuronal function in GO analysis. CX3CL1 over-expression also fully reversed the key upstream cytokine transcription levels that are upregulated by CCI in WT mice in IPA analysis. Most CCI-induced DEGs (2,854) overlap between WT and CX3CL1-Tg mice. CX3CL1 over-expression significantly reversed the key processes seen in WT-CCI mice. **Conclusion:** We concluded that enhancing CX3CL1 signaling significantly mitigated neuropathic pain and dramatically normalized nerve injury induced DEGs that regulate a wide range of neuroimmune functions.

Poster #33

Ankit Uniyal, Ilyas Berhane, Chi Zhang, Qin Zheng, Niyada Hin, Ajit G. Thomas, Jing Liu, Qian Huang, Xiang Cui, Qi Peng, Barbara S. Slusher, Srinivasa N Raja, Xinzhong Dong, Takashi Tsukamoto, Yun Guan

Orally active positive allosteric modulators of human Mas-related G protein-coupled receptor X1 as novel therapeutics for neuropathic pain

Objective and rationale: Mas-related G protein-coupled receptor C (MrgprC, human MrgprX1) is expressed specifically in small-diameter primary sensory neurons and is a promising new analgesic target. Positive allosteric modulator (PAM) has the advantage over the orthosteric agonist in terms of safety and selectivity by promoting spatial and temporal GPCR signaling dependent upon endogenous ligand availability. Here, we aim to develop new orally active human MrgprX1 PAMs and determine their efficacy and safety in mouse models of neuropathic pain. Methods: We have generated a BAC-transgenic mouse line in which MrgprX1 is expressed under the control of the mouse MrgprC promoter.

We then crossed this line into the Mrgpr -/- background, to generate MrgprX1:Mrgpr-/- mice (MrgprX1). In this way, only human MrgprX1 is expressed in mouse primary sensory neurons. We also identified a submicromolar MrgprX1 PAM, 6-(tert-butyl)-5-(3,4-dichlorophenyl)-4-(2-(trifluoromethoxy)phenoxy)thieno[2,3-d]pyrimidine (BDTTP). Results: *In-vitro* assays demonstrated that BDTTP has an EC50 of 0.1 µM with half-lives of >30 min and >60 min in mouse and liver microsomes, respectively. Oral pharmacokinetic studies in mice suggested that BDTTP is orally available with a spinal cord-to-plasma ratio of 13%. Cmax in the spinal cord is more than 40-fold greater than the EC50 value of BDTTP. *In-vivo* efficacy studies showed that BDTTP (100 mg/kg, p.o.) inhibited heat hypersensitivity (Hargreaves test) in humanized MrgprX1 mice, but not in $Mrgpr^{-/-}$ mice, after nerve injury. The peak effects were observed at 2-hour post-administration. Spontaneous ongoing pain behavior (flinching, licking, and shaking) after nerve injury was also inhibited by BDTTP in MrgprX1 but not in $Mrgpr^{-/-}$ mice. An acute *in-vivo* toxicity study using single-time administration of the compound (100 mg/kg, p.o.) did not elicit any behavioral abnormalities in MrgprX1 mice (e.g., sedation, itch scratching, agitation). The open-field and rota-rod tests suggested that BDTTP did not produce any CNS-associated side effects (impaired locomotor or motor coordination). Conclusion: Our study suggests that orally active MrgprX1 PAMs could pave the way for the development of a novel class of effective non-opioid pharmacological agents for the management of neuropathic pain with minimal side effects.

HEAL Biomarker Program

Poster #32

Anukriti Sharma, Ken B. Johnson, Bihua Bie, Courtney E. Hershberger, Yuri Kida, Emily E. Rhoades, Jennifer Hockings, Mei Wei, G. Thomas Budd, N. Lynn Henry, Charis Eng, Joseph Foss, Daniel M. Rotroff

Developing multi-omic biomarker signatures for chemotherapy-induced peripheral neuropathy (CIPN) in patients with breast cancer

Taxanes are a commonly used class of chemotherapeutics used to treat a variety of solid tumors, including breast cancers. However, up to 70% of patients treated with taxanes experience chemotherapy-induced peripheral neuropathy (CIPN). In up to 20% of patients, CIPN is severe enough to impact quality of life both during and after treatment. CIPN often presents as tingling, numbness, or burning in the hands and feet and can result in irreversible peripheral nerve dysfunction and it is one of the most common causes of treatment discontinuation, potentially impacting clinical outcomes. The mechanisms underlying CIPN are poorly understood, which has prevented the development of effective treatment options. No tools exist to predict which patients will likely develop CIPN during taxane treatment. To address the clinical gap in identifying patients at risk of CIPN, we initiated the Genetics and Inflammatory Markers for CIPN Study (GENIE) study, a multi-omic assessment of genetic and inflammatory markers of CIPN, as part of the National Institutes of Health (NIH) Helping to End Addiction Long-term (HEAL) initiative (https://heal.nih.gov/). We are using machine learning to build predictive biomarker signatures that identify patients at increased risk of developing moderate-to-severe CIPN during taxane treatment. Using pre-treatment, on-treatment, and post-treatment blood samples from 350 patients to date with breast cancer treated with taxanes, we are investigating genetic, transcriptional, epigenetic (DNA-methylation), protein, and metabolic associations with validated self-reported pain questionnaires that measure sensory, motor, and autonomic symptoms, and functional limitations related to CIPN. We have determined that multi-omic machine-learning is able to predict those at risk of CIPN using pre-treatment blood samples and questionnaires with 83% accuracy, outperforming individual modalities. Furthermore, multi-omic pathway analysis is revealing putative mechanisms by which taxanes may cause neuropathic symptoms. Overall, this preliminary analysis highlights the value of incorporating multiple types of molecular markers to improve overall predictions of complex diseases and demonstrates the potential for this approach to identify which patients are at risk of CIPN.

Poster #31

Mikhail I. Nemenov, Jordan Zhang Michael Klukinov, Chelsea Allen, Chelsea Allen, David Yeomans, Cathy Revera, Miguel Numa, J. Robinson Singleton

Biomarker of Peripheral Neuropathic Pain and Response to Therapy Based on Sensory and Pain Thresholds of Aδ and C Nerve Fibers and Flare Evoked by Diode Laser

Traditional quantitative sensory tests (QST) have struggled to effectively correlate with neuropathic peripheral pain or differentiate between patients with painful and painless neuropathy[1]. Recent advances in diode laser fiber selective stimulation (DLss) have shown promise in addressing these limitations[2]. Unlike traditional QST, DLss is not sensitive to age differences and can distinguish between painful and painless neuropathy[3]. At the pain threshold, DLss stimulation induces an axon reflex flare linked to the activation of C mechano-insensitive (CMi) fibers, which are pivotal in painful neuropathy[4].

The development of a reliable biomarker reflecting the pathogenic mechanism of neuropathic pain is crucial for analgesic clinical trials and pain diagnostics. The DLss technique, while promising, requires comprehensive validation as a response biomarker. This presentation aims to describe the DLss method, utilize flare recording to confirm the action of DLss through CMi fibers, and assess test-retest reliability in healthy subjects, using warmth detection thresholds (WDT) and heat pain detection thresholds (HPT) as controls.

Eleven healthy subjects aged 25 to 67, with no history of neuropathic or musculoskeletal pain, chronic analgesic use, or diabetes, participated in the study. Each volunteer underwent multiple evaluations with at least a 48-hour gap between testing days.

For detection and pain thresholds, a grid on the foot dorsum was marked, and laser sensory detection and pain thresholds for Aδ and C fiber stimulation were quantified using the "method of levels." Stimulation started below detection intensity and increased incrementally until the participant reported pain between 3 and 4 on a 0–10 numerical pain rating scale. This was repeated three times during each session to calculate the average thresholds.

DLss-evoked flare was induced using the amperage of C fiber stimulation pain thresholds stimulation. Blood flow around the stimulated area was recorded using Speckle Imager (Perimed, Sweden) and temperature measurements were taken to determine the flare's characteristics.

Warm detection thresholds (WDT), cold and heat pain thresholds (HPT) were determined using Q-Sense (Medoc, Israel). A Peltier thermode with a 9.0 cm2 contact area was applied to the test site, and all thresholds were determined using a standard algorithm. This involved continuous temperature ramping from 32°C to the subject's stop signal. The average threshold was calculated from three measurements.

Results showed that axon reflex flare was observed at or below the pain threshold for all volunteers. The average group percent differences between testing sessions for C fiber pain threshold, Aδ fiber detection, and Q-Sense HPT were 7.1%, 11.0%, and 6.6%, respectively. The modified flare method demonstrates promise in terms of Flare Area reproducibility, with percentage differences below 10%, but further testing is needed to confirm its reliability in all subjects.

These findings indicate the potential of DLss as a reliable biomarker for neuropathic pain, with significant advantages over traditional QST, particularly in terms of age sensitivity and its ability to differentiate between painful and painless neuropathy. Further research and validation of DLss as a response biomarker in clinical trials and pain diagnostics are warranted.

Acknowledgement: NIH Heal Initiative/NINDS grant # R61NS122298

HEAL Initial Translational Efforts in Analgesic Development

Poster #30

M. Imad Damaj, Ph.D., Bryan Mckiver and Devanand Sarkar, Ph.D.

The multifunctional protein astrocyte elevated gene-1 (AEG-1) plays an essential role in chronic inflammatory and neuropathic pain

Rationale: Current treatments for chronic pain have been observed to have low efficacy in treating chronic pain, produce severe adverse effects, or high abuse liability. It has become quite clear that there is a vital need to develop more efficacious, non-opioid, treatments for chronic pain. Astrocyte elevated gene 1 (AEG-1) is a multifunctional protein shown to play a critical role in inflammation and multiple intracellular signaling pathways. The role of AEG-1 in cellular inflammation appears to be primarily facilitated by its direct interaction with the transcription factor NFκB, a key transcriptional regulator of pro-inflammatory cytokine (PIC) expression. Two primary underlying causes of chronic pain are inflammation and nerve damage. Additionally, inflammatory cytokines and chemokines have been shown to play an important role in pre-clinical models of both chronic inflammatory pain and neuropathic pain. Therefore, we hypothesize that AEG-1, as a key regulator of inflammation, may play a role in the development and maintenance of chronic pain. Objectives: To investigate if AEG-1 contributes to the development of chronic inflammatory and neuropathic pain, making it a potential therapeutic target for these conditions.

Methods: Adult AEG-1 wild type (AEG-1 WT), global knockout (AEG-1 KO), AEG-1 floxed (AEG-1fl/fl), and AEG-1 myeloid conditional KO (AEG-1ΔMAC) male and female mice on C57BL/6J background were investigated in 2 mouse models of chronic pain: Chronic inflammatory pain (intraplantar injection of 50% CFA) and Chemotherapy-Induced Peripheral Neuropathy (CIPN) induced via four intraperitoneal injections of paclitaxel (PAC) at a dose of 8 mg/kg. Mechanical, thermal and cold were assessed. qRT-PCR was used to assess the mRNA expression levels of AEG-1 and PIC in the lumbar (L4-6) dorsal root ganglia (DRG) collected from mice injected with either CFA or PAC. In addition, electrophysiological and morphological parameters of the disease were measured.

Results: In our model of CFA-induced chronic inflammatory pain, AEG-1 KO and AEG-1ΔMAC mice displayed protection from CFA-induced mechanical hypersensitivity, thermal sensitivity, and reduced paw edema compared to AEG-1 WT and AEG-1fl/fl counterparts, respectively. In our model of CIPN, AEG-1 KO mice displayed protection from PAC-induced mechanical hypersensitivity and cold sensitivity as well as decrease in caudal nerve conductance and Intraepidermal nerve fiber density (IENFs) compared to WT counterparts. Plasma concentrations of PAC did not differ between AEG-1 KO or WT mice at either 1 or 8 hours post injection. AEG-1 deletion reverses both CFA and PAC-induced neuroinflammation in the DRGs of male mice compared to their WT counterparts. A similar protection was seen in the AEG-1 myeloid conditional KO (AEG-1ΔMAC) mice after paclitaxel.

Conclusions: Our data suggest that AEG-1 expression in myeloid immunome cells mediates a neuroinflammatory response that contributes to the development of inflammatory pain and CIPN associated pain-like behaviors in mice.

Innovation Grants to Nurture Initial Translational Efforts (IGNITE) Program

Poster #17

Aniruddha Karve, Bhavesh Gabani, Siddarth Gadgil, Shravani Kulkarni, Gary Gudelsky, Pankaj B. Desai

Translational research to facilitate development of novel therapeutic combinations of letrozole for the treatment of glioblastoma: Part 1- investigation with PARP inhibitors

Background: Based on ongoing pre-clinical/translational research, letrozole (LTZ) a third-generation aromatase inhibitor, appears to be a promising novel therapeutic for the treatment of glioblastoma (GBM). It synergistically enhances the efficacy of the DNA alkylating agent temozolomide, one of the few approved chemotherapeutics for GBM, against patient-derived GBM cells. RNA-seq analysis of the LTZ treated tumor samples obtained from the ongoing "window of opportunity" phase 0/1 dose escalation clinical trial revealed that LTZ downregulates several genes encoding for DNA-damage repair proteins such as BRCA2 in a dose dependent manner.

Hypothesis: Our central hypothesis for this project is that combination of LTZ with targeted therapeutic agents that enhance DNA damage results in synergistic effects against GBM. The objective of this study is to evaluate the effects of poly-ADP ribose polymerase (PARP) inhibitors in combination with LTZ against patient-derived GBM cells. Our secondary aim for this project is to delineate the blood-brain barrier (BBB) permeability of μPARP inhibitors.

Methods: Employing patient derived G43, G75 and JHH-136 GBM lines we assessed the influence of PARP inhibitors (olaparib, pamiparib, veliparib and niraparib) in combination with LTZ on cell viability and neurosphere growth. In addition to the *in vitro* experiments, we also utilized male CD-IGS rats (n=6; body weight, 250g) to assess systemic and brain pharmacokinetics (PK) of pamiparib and niraparib using cerebral microdialysis.

Results: Treatment of the GBM cells with LTZ and PARP inhibitors resulted in synergistic cytotoxic effects in all GBM lines used for this study. Treatment in combination with LTZ (0.05 μM), a non-cytotoxic concentration of LTZ in these lines significantly decreased the IC50 values for PARP inhibitors (the IC50 values for niraparib, veliparib, pamiparib and olaparib combinations from LTZ ranged from 0.17 – 0.58 μM, 0.2 -1 μM, 0.08 to 0.01 μM and 0.65 – 2.1 μM, respectively). Combination Index analysis suggested that LTZ synergistically potentiated the activity of these PARP inhibitors. Furthermore, the systemic and brain PK analyses of pamiparib and niraparib indicated that the partitioning of the drug from plasma to the brain extracellular fluid (ECF) measured as the ratio of the area under the curve (AUC) of the unbound ECF and plasma concentrations (Kpu,u) values were 0.98 and 0.45, respectively.

Conclusions. Our studies provide a strong foundation for pursuing further development of combination therapy of GBM with LTZ and PARP inhibitors based on the observed synergistic potentiation of cytotoxic effects in patient-derived GBM lines and excellent BBB permeability of pamiparib and niraparib. Based on a matrix-based quantitative evaluation it appears that these PARP inhibitors meet our cut-off criteria for further pre-clinical evaluation in patient-derived xenograft animal models. Additional studies with other targeted agents such as CDK4/6 and HDAC inhibitors are similarly in progress. By the end of Year 2 of the study at least six compounds will be rank-ordered and evaluated as combination partners with LTZ for assessment in patient-derived xenograft animal models.

Poster #18

Edwina Abou Haidar, Shilpa Prabhakar, Pike See Cheah, Roberta L. Beauchamp, Anat Stemmer-Rachamimov, Vijaya Ramesh, Casey A. Maguire, Xandra O. Breakefield

Comparison of therapeutic efficacy of gene therapy for tuberous sclerosis type 2 with standard of care everolimus

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder caused by a hereditary loss of function mutation in one of two tumor suppressor genes, *TSC1* and *TSC2*, encoding for hamartin or tuberin respectively. These proteins form a complex that constitutively inhibits the mammalian target of rapamycin (mTOR) signaling pathway. In TSC-related lesions, the loss of either proteins due to a somatic mutation in the normal allele in susceptible tissues causes over activation of mTOR signaling, subsequently leading to cellular proliferation and overgrowth in many vital organs, most commonly affecting the brain, kidneys, skin, heart and lung. Neurological features of the disease include seizures, cognitive impairment and autism. We have recently demonstrated in a mouse model, that gene therapy using an adeno-associated virus (AAV) vector carrying a "condensed" form of human tuberin (cTuberin) is a promising therapeutic strategy for TSC2. Here, we compare our gene therapy strategy to the current standard of care for TSC patients, the mTOR inhibitor everolimus. A mouse model of TSC2 generated by AAV1-Cre recombinase disruption of homozygous Tsc2-floxed alleles at birth (P0) via intracerebroventricular injections has a shortened lifespan (mean 50 days) and brain pathology consistent with TSC, including proliferation of ependymal/subependymal layer of the lateral ventricles. When these mice were then single injected intravenously at post-natal day 21 (P21) with an AAV9 vector encoding cTuberin, most survived for more than 150 days. Further immunostaining analyses in this model showed that AAV9 vector transduced cells throughout the brain, including subependymal cells, astrocytes, and neurons. Post treatment neuropathologic assessment resulted in a reduction in ependymal/subependymal cell proliferation and reduction in phosphorylation of ribosomal protein S6 . Interestingly, continuous treatment with everolimus, used in TSC patients, extended survival for up to 74 days but failed to maintain life after discontinuation. These studies demonstrate the potential of treating life-threatening TSC2 lesions with a single intravenous injection of AAV9-cTuberin as compared to the alternative drug treatment currently in use clinically.

Poster #56

Yunlu Zhu, Paige Leary, Qing Bai, Edward A. Burton, David Schoppik

Linking molecular abnormalities to behavioral deficits using a zebrafish model for tauopathies

Tauopathies are neurodegenerative diseases characterized pathologically by accumulation of abnormal Tau in the brain. However, it remains unclear how these molecular and cellular dysfunctions lead to behavioral deficits, especially during the early stages of pathogenesis. To dissect disease mechanisms across multiple biological scales, we generated a zebrafish model of progressive supranuclear palsy (PSP), a primary tauopathy causing unexpected falls in patients early in disease progression, by expressing human 0N/4R-Tau in the evolutionarily conserved vestibulospinal (VS) nucleus. Human Tau-expressing zebrafish exhibit impaired balance control during free-swimming while maintaining normal locomotor ability compared to their siblings. Functional imaging of the VS nucleus shows decreased calcium signals in Tau-expressing neurons in response to tilt stimulus. Interestingly, we also observed ectopic accumulation of acidic organelles in the cell bodies of Tau-positive neurons, suggesting abnormal lysosomal function. Taken together, our zebrafish PSP model allows us to understand molecular and cellular mechanisms of balance deficits in tauopathies and can be a powerful system for preclinical drug screening and evaluation of potential therapeutic targets.

Poster #35

Luiz Ferrari, Gary Donaldson, Ashley Wilkinson, Norman Taylor

Development of a fibromyalgia analog model index to comparatively evaluate the face and construct validities of various rat models of fibromyalgia

Millions of Americans suffer from fibromyalgia syndrome (FMS) and experience severe disability and diminished quality of life, making it a significant public health problem. The goal of this project was to develop and validate a multivariate regression index in rats that will mimic current clinical fibromyalgia indices. We hypothesize that this index will improve the evaluation of face and predictive validities of animal FMS models, and provide a defined method to compare them. We used the index to compare the established reserpine-injection model of FMS and an innovative model, the Dahl salt-sensitive (SS) rat. Male (n=30) and female (n=30) rats were used; FMS-like symptoms were induced in Sprague Dawley (SD) rats by injection of reserpine. Six behavioral endpoints were tested, in the same individual, for FMS traits: behavioral aspects of fatigue (24-h home cage distance traveled), muscle tenderness (bilateral evaluation of mechanical nociceptive threshold in the gastrocnemius muscle), widespread pain (hind paw and facial withdrawal thresholds), anxiety (zero maze test), and depression (forced swim test). The results were analyzed using regression modelling within a rigorous multivariate framework to define relationships in observable clinical phenotypes to develop the Fibromyalgia Analog Model (FAM) index. The data was used to maximize the internal validity of the measurements. Five statistical milestones were considered in evaluation of the FAM index including measures of model fit (Root Mean Squared Error of Approximation = 0.039), variance inclusion (R2= 0.564), reliability (rjj ≥ 0.7 for all 6 tests along with a factor score determinacy coefficient = 0.98), and robustness (Cohen's d = 2.4). We certified the external validity of the FAM index using two additional strains, SS (both sexes, n=30/group) and saline-treated SD rats (both sexes, n=30/group). The analogous model for the SS rats also converged (RMSEA = 0.109) while the one-factor FAM model did not converge for the saline-treated SD rats. This indicates that one factor does not adequately explain the covariation in saline-treated SD rats. This is according to our twofold prediction that the model would fit well in reserpine-treated but not in saline-treated rats. The hypothesis that reserpine treatment induces a central organizing mechanism for objectively different behavioral tasks (analogous to fibromyalgia) is therefore supported. Additionally, the FAM model also identified the SS rat as a promising FMS model. There is a provisional suggestion that future work with this population could yield an even better fit and index with the FAM model.

Poster #57

Piyali Guhathakurta, Sarah A. Denha, Amanda R. Keller, Anna L. Carter, Alexandra Atang, Robyn T. Rebbeck, Bengt Svensson, David D. Thomas, Thomas S. Hays and Adam W. Avery

Early-phase drug discovery of β-III-spectrin actin-binding modulators for treatment of spinocerebellar ataxia type 5

β-III-spectrin is a key cytoskeletal protein that localizes to the soma and dendrites of cerebellar Purkinje cells, and is required for dendritic arborization and signaling. Mutations in the *SPTBN2* gene encoding β-III-spectrin cause the neurodegenerative disorder spinocerebellar ataxia type 5 (SCA5). Currently, there is no cure or therapy for SCA5. Numerous SCA5 mutations localize to the β-III-spectrin N-terminal actin-binding domain (ABD). We previously showed that an ABD-localized *L253P* mutation causes a 1000-fold increase in actin-binding affinity. Here we report the molecular characterization of ten additional ABD-localized mutations, and our progress towards developing a SCA5 therapeutic. Significantly, we found that increased actin binding is a shared molecular consequence of all tested SCA5 mutations. Further we developed a novel fluorescence-based high throughput screening platform that uses purified *L253P* β-III-spectrin ABD protein and F-actin. To validate the assay, we screened a 3,000-compound library of FDA

approved drugs. Significantly, numerous compounds were identified that decreased FRET between fluorescently labeled *L253P* ABD and actin. The activity of multiple Hit compounds was confirmed in orthologous co-sedimentation actin-binding assays. Our results suggest that a small molecule that reduces actin binding of mutant β-III-spectrin may be broadly effective as a SCA5 therapeutic. Future medicinal chemistry to optimize our current Hit compounds, and additional screening of larger compound libraries, may lead to a SCA5-specific therapeutic.

Poster #21

Ronald Gaykema, Aleksandra Maciejczuk, Madison Failor, Edward Perez-Reyes
Validation of a pharmacokinetic-based pilocarpine model of temporal lobe epilepsy

Our long-term goal is to develop novel therapeutics for epilepsy patients whose seizures are not well-controlled by current drugs (pharmacoresistant). Many of these patients have a type of focal epilepsy called temporal lobe epilepsy (TLE). There has been little progress in the development of novel therapies for these patients because of the lack of suitable animal models. Current TLE models show a highly variable occurrence of seizures that precludes drug testing. The proposed studies will validate the usefulness of new mouse model of TLE that overcomes these problems. It was discovered that a specific strain of mice, VGAT-Cre, are susceptible to developing epilepsy. These mice express Cre recombinase under the control of the vesicular GABA transporter (*VGAT*), a gene that is specifically expressed in GABAergic inhibitory neurons. Loss, or dysfunction, of these neurons in the hippocampus has been linked to the development of temporal lobe epilepsy.

We report here the results from NS112549 “Validation of a Novel Mouse Model of Temporal Lobe Epilepsy” (PI: Edward Perez-Reyes) The goal of this grant was to improve the seizure patterns of electrically-kindled VGAT-Cre mice. By combining the chemoconvulsant kainate, along with electrical kindling, we were able to improve the spontaneous seizure frequency significantly. Unexpectedly, we discovered that their epilepsy remits after 3 weeks. This sort of an epilepsy duration makes it very difficult to screen for novel therapeutics.

We now present studies with pilocarpine treatment of VGAT-Cre mice that produce the “Holy Grail” of seizure patterns: spontaneous seizure frequency > 2 per day, seizures every day with little-to-no seizure-free days, and that maintains these features for greater than 4 weeks. We have applied again to the IGNITE program to develop and validate this “PK-Pilo” model of TLE. This grant would also fund a drug screening campaign by Drs. Karen Wilcox and Cameron Metcalf at the University of Utah, who also direct the NIH-sponsored Epilepsy Therapy Screening Program.

Poster #29

Jonathan R. Weinstein, Mitzi Adler-Wachter, Brendan Schweitzer, Ashley McDonough, Ruth D. Lee, Yi-Je Chen, Heike Wulff
Repurposing the K_{Ca}3.1 inhibitor senicapoc for treatment of acute ischemic stroke

Background: Acute ischemic stroke (AIS) is a leading cause of death and long-term disability. Both microglia (MG) and macrophages (MP) are critical effector cell types in ischemic brain injury. K_{Ca}3.1 is a calcium-activated potassium channel that is upregulated in reactive MG and MP. Studies using genetic deletion or pharmacological inhibition of K_{Ca}3.1 demonstrate this channel is critical for pro-inflammatory activation of MG/MP and exacerbation of stroke pathophysiology. Senicapoc is a K_{Ca}3.1-specific inhibitor that has been used in human clinical trials for non-neurological indications and was proven safe. Here we evaluate the potential for repurposing senicapoc for AIS. **Methods:** Young adult male mice

underwent 60 min middle cerebral artery occlusion (MCAO)/reperfusion. Senicapoc's pharmacokinetic (PK) profile was determined using HPLC/mass spectroscopy. Drug levels in plasma and brain were quantified at multiple time points. Effects of senicapoc on post-stroke release of cytokines/chemokines was determined by multiplex ELISA. Inflammatory infiltrates were quantified with immunofluorescent microscopy (IFM). Efficacy studies included: (i) infarct volume (MRI T2 and IFM), white matter integrity (MRI DTI) and longitudinal neurobehavioral (NB) outcomes. NB studies included grid test and alternating T-maze. *In-vitro* chromogenic assay was used to assess senicapoc's effect on proteolytic activity of tissue plasminogen activator (tPA). **Results:** Administration of senicapoc (40 mg/kg, i.p.) twice daily for 7 d starting 12 h after MCAO resulted in ~55% reduction in infarct volume with corresponding improvements in NB outcomes. Free senicapoc levels in brain ranged from 20 – 200 nM in stroked mice at 1, 4 & 12 hours post administration (exceeding senicapoc's IC50 (11 nM) for $K_{Ca}3.1$). Senicapoc attenuated stroke-induced: (i) infiltration of MG/MP and T-cells and (ii) upregulation of IL-1b, TNFa and IFNg. Senicapoc had no effect on tPA activity. **Conclusions:** We provide proof-of-concept data that senicapoc, administered in an extended temporal window, can reduce infarct volume, improve NB outcomes and attenuate stroke-induced neuroinflammation. Senicapoc has a favorable PK profile, good CNS penetration and no effect on tPA.

Poster #58

Grant L. Austin, Matthieu Colpaert, Pankaj K. Singh, Manuela Corti, Barry J. Byrne, Ramon C. Sun, Craig W. Vander Kooi, Dustin Armstrong, and Matthew S. Gentry

Antibody-enzyme fusions to treat neurological glycogen storage diseases

Lafora disease (LD) is an autosomal recessive neurological glycogen storage disease that manifests as progressive myoclonic epilepsy and childhood dementia. Symptom onset is in the second decade of life and patients usually succumb to the disease within a decade of symptom onset. There are currently no effective treatments for LD. LD patients and mouse models accumulate aberrant cytoplasmic glycogen-like aggregates called Lafora bodies (LBs) in cells from most tissues, including the brain, which cause the underlying metabolic changes resulting in LD epilepsy, neurodegeneration, and brain inflammation. Herein, we report development of an antibody-enzyme fusion as a pre-clinical treatment for LD that ablates the pathologic LBs and normalizes brain metabolism. The antibody-enzyme fusion, VAL-1221, combines the cell penetrating capabilities of the 3E10 antibody fab fragment with acid, alpha-glucosidase (GAA). We demonstrate that VAL-1221 can degrade LBs purified from two LD mouse models *in vitro*, penetrate cells and tissues *in vivo*, and degrade systemic LBs when delivered intravenously. Furthermore, we show that when delivered via intracerebroventricular administration, VAL-1221 ablates LBs in LD mouse model brain, restores brain metabolic profiles, and reestablishes brain N-linked glycosylation patterns that are perturbed in LD. This work establishes that VAL-1221 is an effective preclinical treatment for LD and suggests efficacy for other neurological glycogen storage diseases.

Poster #59

Piyush Padhi, Ileia J. Scheibe, Alyssa A. Otto, Jacob P. Thomas, Ahyoung Jang, Nick Backes, Gary Zenitsky, Huajun Jin, Vellareddy Anantharam, Arthi Kanthasamy, Karin Allenspach, Jonathan Mochel, Gregory J Phillips, and Anumantha G. Kanthasamy

Engineered programmable, microbiome-based levodopa live-bacterial therapeutic achieves highly favorable pharmacokinetics and pharmacodynamics and improves motor and non-motor symptoms of Parkinson's disease without levodopa-induced dyskinesia in a preclinical model of progressive dopaminergic neurodegeneration

As the gold-standard dopamine replacement therapy for Parkinson's disease (PD), levodopa/benserazide tablets have significant drawbacks associated with chronic oral administration, including the

development of L-DOPA-induced dyskinesia (LID) and 'ON-OFF' motor complications. To overcome the limitations of non-continuous, pulsatile delivery of L-DOPA imposed on the brain by repeated fixed doses, we bioengineered a novel human probiotic, *E. coli* Nissle 1917 (EcN), that continuously produces and dose-dependently releases L-DOPA in the gut. By utilizing genome engineering and synthetic biology techniques, we successfully integrated a recombinant *hpaB/C* gene into the EcN chromosome, allowing for the conversion of L-tyrosine to L-DOPA. Our lead levodopa bacterial live-biotherapeutic (LDBL), EcNL-DOPA, achieved favorable pharmacokinetics, pharmacodynamics, and gastrointestinal (GI) kinetics when administered orally twice daily, resulting in steady and non-fluctuating dopamine supplementation to the brains of both rodents and canines. To comprehensively evaluate the therapeutic efficacy of EcNL-DOPA compared the current standard-of-care (SOC), we conducted extensive preclinical assessments in MitoPark PD rodent model of progressive degeneration. Our evaluation included phenotypic assessments of gross and fine motor coordination, gait, and GI function after 12 weeks of chronic administration of EcNL-DOPA with benserazide. Additionally, we examined neurochemical, microbiome, GI, and brain toxicological alterations with plasma metabolome, fecal metagenome, gut inflammatory and histological metrics. Our findings suggest that the sustained, non-pulsatile delivery of L-DOPA by EcNL-DOPA improves both gross and fine motor skills over longer period as compared to conventional L-DOPA. Notably EcNL-DOPA MitoPark displayed greater horizontal activity score in generalized locomotor activity assessment and exhibited delayed latency to fall in Rotarod test. Furthermore, EcNL-DOPA improved step sequence regularity index and cadence gait measures in MitoPark using CatWalk gait analysis. While also enhancing motor function, chronic EcNL-DOPA administration was well tolerated and improved gastrointestinal function, including gastrointestinal transit time and intestinal permeability. Importantly, we next evaluated whether EcNL-DOPA prevented the generation of LID in the MitoPark after 8 weeks of chronic administration compared to SOC. Through blinded analysis of the cylinder test, EcNL-DOPA ameliorated hind-paw and three-paw dyskinesia compared to the conventional chemical L-DOPA therapy. We further validated these findings by assessing the well-established molecular markers of LID, including αFosB, pDARPP-32, and pERK. Overall, our engineered LDBL represents a novel L-DOPA treatment modality that ensures long-term effectiveness with minimal side effects, distinguishing it from conventional anti-Parkinsonian therapeutic approaches. (NIH R61NS112441, U01AG074960-01A1).

Poster #75

Rajesh Amin, Ian Steinke, Fajar Setyo Wibowo, Sieun Yoo, Vishnu Suppiramaniam, Meenakshi Singh
Evaluation of a specific LXR/PPAR agonist for treatment of Alzheimer's disease

The increased incidence of Alzheimer's disease (AD) and its associated mortality rate represent an unmet medical need and critical need for novel molecular therapeutics. Recent work focusing upon patients with 1 or 2 apoE4 alleles has highlighted the association of brain cholesterol dysregulation with elevated pathological burden, and neurodegeneration. These studies have highlighted the importance of the nuclear receptor Liver X receptor (LXR) for developing AD therapies. However, LXR agonists have been observed to induce hepatotoxicity and neutropenia in humans and thus have failed clinical trials for atherosclerosis. Our newest dual agonist, AU403, was developed computationally as a novel Liver X Receptor-β (LXRβ) with partial PPARδ/α activity. Our library of compounds (>100) was created in silico and is based upon selective amino acid interactions in the ligand-binding domains of both LXRβ and PPARδ/α with negligible PPARγ activity. Thus our design serves as a dual LXRβ/ PPARδ/α agonist. This strategy seeks to avoid the unwanted side effects of traditional LXRα and PPARγ agonists, including liver toxicity, neutropenia, edema, and heart failure. Methods: Our lead compound AU403 was designed computationally using Schrodinger software and molecular dynamics to model enzymatic activity against known PPAR agonists, using crystal structures of PPAR and LXR nuclear receptors. Further

AU403, drug design was optimized by modeling ADME properties using both QikProp and GastroPlus to enhance distribution to the CNS. Improved working declarative memory was observed in 12 month 3xTgAD mice, using Y-maze and Novel object recognition studies after 3 months of AU403 treatment (5mg/kg / daily). Improvement in synaptic plasticity was measured by long-term potentiation studies. Results: Our in silico design was formulated on Based on these evaluations our lead compound AU403 displays avoidance of Trp443 and Leu439, which are deep in the LXRα-AF2 ligand binding pocket. Furthermore, we observed significant improvement in working declarative memory and long-term potentiation in 12-month old 3xTgAD mice treated with our lead compound AU403. Mechanistically we observed enhanced microglia interaction with dendritic spines density

Conclusion: We have observed that AU403 can serve as a highly novel potential therapeutic agent for AD.

NIH Countermeasures Against Chemical Threats (CounterACT)

Poster #7

José Luis Marrero-Valentin, Pedro A. Ferchmin, Nadezhda Sabeva

4R-cembranoid improves anxiety-like behavior and cognitive deficiencies in the Gulf War Illness mouse model

Persistent neuroinflammation, cognitive dysfunction, and anxiety-like behavior are the primary CNS symptoms in veterans suffering from Gulf War Illness (GWI). Despite the advancements in research, effective treatment against GWI has not been established. This study investigated the efficacy of α7nAChR modulator, the 4R-cembranoid (4R), for improving cognitive function and anxiety behavior in a mouse model of chronic GWI. As an anti-apoptotic and anti-inflammatory compound, 4R is known to easily cross the blood-brain barrier (BBB) to protect the brain cells after acute exposure to a surrogate of Sarin, diisopropyl fluorophosphate (DFP).

To recreate GW conditions, we administrated PB and PER with DEET, traces of DFP, and moderate stress for 12 consecutive days in C57BL/J6 male mice. One month later, mice were treated with vehicle or 4R (6 mg/ kg) for four weeks. Behavioral tests revealed cognitive deficits and anxiety present in GWI mice receiving vehicles. GWI mice treated with 4R improved their spatial learning in a two-day Barnes maze test (BM) akin to the control-vehicle group, measured by latency to escape box. Conversely, GWI-vehicle retained the same latency throughout all training sessions. Nevertheless, 48 hours after BM training, all animals reached the escape hole with similar latency, suggesting that GWI mice have learning memory deficiency that can be overcome with time.

To evaluate the animals' ability to adapt to a changing environment, we removed the escape box and conducted four consecutive probe tests (P1-P4) spaced by 24 hours. GWI mice had a delayed adjustment to the changing environment and maintained the same exploratory nose-poke pattern for the non-escape holes (errors) during the four probe tests. Conversely, GWI-4R and the control groups (vehicle and 4R) demonstrated awareness at P1 that the escape box is missing and assessed the escape-hole and non-escape-hole with similar rates (P1-P4). Our data suggests that 4R treatment can improve long-term memory retention and memory extinction in GWI.

To confirm that 4R alleviates cognitive dysfunction in GWI, we employed a different behavior test where the animals' ability to detect subtle changes in the environment was measured by minor changes in the location of an object (Object Location Recognition test). GWI mice that received vehicle displayed impairment in this cognitive task and spent equal time with the objects in the novel and familiar place. In contrast, GWI mice that received 4R spent significantly higher percentages of their exploration time with the object in the novel location, similar to the performance of the control groups.

Lastly, GWI mice treated with 4R had ameliorated anxiety-like behavior measured with Elevated Plus Maze (EPM). Similar to the controls, the GWI-4R group spent less time in the closed arms and remained 50% longer in the open arms compared to the GWI-vehicle. Moreover, latency to open arms was decreased considerably in GWI-4R, suggesting that the early onset of anxiety in GWI can be corrected via α7nAChR modulation. In conclusion, 4R is a promising candidate for treating GWI veterans and future victims of similar civilian or military adverse events.

Poster #8

Donna A. Nguyen, Marcio de Araujo Furtado, Jerome Niquet, Emily Linz, Caroline R. Schultz1, Michael F. Stone, Claude G. Wasterlain, Lucille A. Lumley

Third generation antiseizure medications lacosamide and rufinamide as an adjunct therapy to midazolam and ketamine against refractory status epilepticus

Status epilepticus (SE) is an emergency condition that requires prompt response to effectively control. The standard first line treatment after SE onset is a benzodiazepine, but protective potency is lost in a time-dependent manner resulting in long-term effects such as neuropathology and epileptogenesis. This development requires the addition of second and third line antiseizure medications (ASMs) to mitigate SE severity. Preclinical models have shown the efficacy of simultaneous administration of ketamine and midazolam in reducing epileptogenesis in a delayed treatment model of cholinergic-induced SE, but with incomplete neuroprotective effects. Third generation ASMs have been of interest in polytherapy models for their safety, tolerability, and lack of sedative effects. Lacosamide and rufinamide are both FDA-approved ASMs with mechanisms focused on voltage-gated sodium channel inactivation. We evaluated the protective effects of midazolam, ketamine, and third generation ASMs lacosamide or rufinamide against cholinergic-induced SE. Adult male rats were exposed to a seizure-inducing dose of an organophosphorous (OP) chemical, which is an inhibitor of cholinesterase, and treated with atropine sulfate and an oxime 1 min later and with midazolam, midazolam-lacosamide, midazolam-rufinamide, midazolam-ketamine-lacosamide, or midazolam-ketamine-rufinamide combination therapies 40 min after seizure onset. Control rats received saline and were treated with midazolam. After exposure, toxic signs were monitored for 5 h and seizure duration and the development of spontaneous recurrent seizures (SRS) were monitored for 14 days. Brain tissue was assessed for SE-induced neurodegeneration, neuronal loss, and loss of GABAergic neurons. Compared to midazolam monotherapy, midazolam-ketamine-lacosamide triple therapy reduced OP-induced behavioral toxic signs, 24 h seizure duration, EEG power integral, incidence of SRS, and loss of GABAergic interneurons. Lacosamide and rufinamide triple therapies reduced the percent change in EEG power bands after treatment, neuronal loss, and weight loss observed in rats treated with midazolam monotherapy. The majority of rats survived in all treatment groups following OP exposure but rats treated with midazolam-rufinamide dual therapy experiencing a 100% survival rate, which was significantly greater than those that received vehicle. Protective effects were observed when lacosamide or rufinamide were administered as adjunct treatments to midazolam and ketamine in a delayed treatment model of OP-induced SE. Differing levels of protection between lacosamide and rufinamide triple therapies were observed with regard to the epileptogenesis, EEG power integral, and brain regions protected from neuropathology, suggesting lacosamide triple therapy may provide better efficacy in protecting from the long-term effects of refractory SE. Overall, both drugs were well tolerated in these combination models and future studies should further explore these two ASMs as a viable treatment against SE.

Poster #9

Marcio De Araujo Furtado, Vassiliki Aroniadou-Anderjaska, Taiza H. Figueiredo, Volodymyr I. Pidoplichko, James P. Apland, Katia Rossetti, Maria F.M. Braga

Preventing long-term brain damage by nerve agent-induced status epilepticus in rat models applicable to infants: significant neuroprotection by tezampanel combined with caramiphen but not by midazolam treatment

Acute exposure to nerve agents induces a peripheral cholinergic crisis and prolonged status epilepticus (SE), causing death or long-term brain damage. To provide preclinical data pertinent to the protection of infants and newborns, we compared the antiseizure and neuroprotective effects of treating soman-

induced SE with midazolam (MDZ) versus tezampanel (LY293558) in combination with caramiphen (CRM), in 12- and 7-day-old rats. The anticonvulsants were administered 1 h after soman exposure; neuropathology data were collected up to 6 months post-exposure. In both ages, the total duration of SE within 24 h after soman exposure was significantly shorter in the LY293558+CRM groups compared with the MDZ groups. Neuronal degeneration was substantial in the MDZ-treated groups but absent or minimal in the groups treated with LY293558+CRM. Loss of neurons and interneurons in the basolateral amygdala and CA1 hippocampal area was significant in the MDZ-treated groups but virtually absent in the LY293558+CRM groups. Atrophy of the amygdala and hippocampus occurred only in MDZ-treated groups. Neuronal/interneuronal loss and atrophy of the amygdala and hippocampus deteriorated over time. Reduction of inhibitory activity in the basolateral amygdala and increased anxiety were found only in MDZ groups. Spontaneous recurrent seizures developed in the MDZ groups, deteriorating over time; a small percentage of rats from the LY293558+CRM groups also developed seizures. These results suggest that brain damage can be long-lasting or permanent if nerve agent-induced SE in infant victims is treated with midazolam at a delayed timepoint after SE onset, while antiglutamatergic treatment with tezampanel and caramiphen provide significant neuroprotection.

Poster #10

R.E. Blair, E. H. Hawkins, R.J. DeLorenzo, L. S. Deshpande

Disease modifying effect of delayed intramuscular atenolol and levetiracetam therapy on cardiac and brain functions following organophosphate induced status epilepticus

Organophosphate (OP) compounds include pesticides and chemical warfare nerve agents. Lethal OP exposure leads to status epilepticus (SE) and survival despite the standard-of-care treatment is associated with cardiac irritability, neuronal injury, chronic memory impairment, and spontaneous recurrent seizures (SRS). We recently showed that treatment with a beta-adrenergic blocker atenolol (AT) and a neuronal calcium-induced calcium release inhibitor levetiracetam (LV) administered after the onset of OP paraoxon (POX)-induced SE significantly reduced mortality. Here, we investigated whether AT+LV would protect the heart and the brain to lower the mortality and neurobehavioral morbidities following POX-induced SE. Male Sprague-Dawley rats were injected with POX (2 mg/kg, *s.c.*). One minute later, atropine (0.5 mg/kg, *i.m.*) and 2-PAM (25 mg/kg, *i.m.*) were injected. At 1-h post SE onset, midazolam (1.78 mg/kg, *i.m.*) was used to terminate SE. Following POX-SE, rats were treated with AT+LV (AT, 5 mg/kg, LV 50 mg/kg, *i.m., b.i.d.* for 7 days) or saline (CON). Separate groups of rats were used for ECG, EEG, behavior, and histopathology. OP intoxication produced rapid SE and increased survival was noted with AT+LV. ECG parameters (QTc and QTd) were significantly increased following POX-SE which were normalized with AT+LV. AT+LV also lowered the cardiac damage index from 2.65 ± 0.19 in POX SE rats to 0.76 ± 0.18 (n= 6, $p<0.001$, Tukey-test). On the Barnes Maze, during the acquisition-phase, latency to escape and the number of errors were significantly lower in AT+LV rats compared to CON and, during the probe trial, AT+LV rats spent significantly more time in the target quadrant and made significantly more visits to the escape zone compared to CON rats. Video-EEG monitoring revealed the presence of POX SE SRS in 73.3% of CON rats while only 56.2% of AT+LV rats exhibited SRS. Additionally, AT+LV resulted in a significant decrease in seizure frequencies from 7.5± 9.6 to 1.7±2.2 SZs/day when compare to CON (n=8, $p\leq 0.01$, t-test). AT+LV treatment resulted in significant neuroprotection in the dentate gyrus hilus when compared to CON represented by an increase to 135.6±8.4 from 103.7±11.2 cells/mm2 respectively (n=11, $p\leq 0.05$, Tukey-test). Our results indicate that decrease in POX-SE mortality following AT+LV could be due to normalization of the QTc prolongation and a reduction in myocardial injury. Our results also indicate AT+LV was neuroprotective, it significantly improved

memory outcomes and lowered SRS occurrence in POX-SE rats. AT+LV therapy could improve survival and provide significantly better neurological outcomes following OP toxicities.

NINDS Translational Biomarker Program

Poster #73

J. Block, M. Awad, J. Olson, J. N. Bentley, M. Holland, S. A. Brinkerhoff, A. Nakhmani, M. Moffitt, H. Walker

A method for stimulus transient removal by leveraging the absolute refractory period: Validation with cathodic versus anodic deep brain stimulation

Neurostimulation generates large electrical artifacts that obscure underlying physiological potentials. Stimulus-evoked recordings are typically "blanked" for 1 to 2.5 ms after pulse onset to remove artifact and enable visualization of the signal after the "blanking" window, but physiological signals present during the "blanking" are unfortunately also subtracted with this technique. We have proposed an alternative method for separating artifact from neural response based on templates and the refractory properties of neurons, and that we hypothesize reveals valuable neural activity within 1 ms of the pulse onset. In the present work, we use the difference in stimulation properties of anodes and cathodes to generate supporting evidence of the effectiveness of our novel artifact removal technique. We studied intracranial electrophysiology in 5 consecutive patients with Parkinson's disease undergoing deep brain stimulation (DBS) surgery as part of routine care. Pairs of monopolar cathodic and anodic stimuli were delivered from the DBS electrode across a range of inter-stimulus intervals (0.3 to 16 ms) and recorded from unused adjacent DBS electrodes. Broadband sampling (100 kHz) and precise synchronization yielded robust event-related potential templates for the stimulus transient during the absolute refractory period. We then subtracted this template waveform across experimental conditions and analyzed the residual signals. These potentials display absolute and relative refractory periods and phase-independence from the stimulus transient. The earliest detectable responses are almost entirely obscured by stimulus artifact and occur at very short latencies with a peak, on average, at 0.28 ms after pulse onset (range of 0.19 to 0.38 ms). Monopolar cathodic and anodic DBS pulses elicit distinct patterns of local tissue activation. In 5 out of 5 of the subjects, cathodic stimuli elicit larger local tissue responses than anodic stimuli at the same amplitude, consistent with known clinical phenomenology (i.e., lower activation thresholds with cathodic stimulation). The early tissue response for cathodic stimulation was, on average, 3.3 times greater than for anodic stimulation (range of 1.3 to 7.2 times larger). This method for stimulus artifact removal improves on prior efforts because it allows direct measurement of local tissue responses without requirements for stimulus polarity reversal (each of which can engage distinct neural elements), template scaling, specialized filters, or other techniques. Future neuromodulation systems could utilize this method or its extensions as a proxy for dose or circuit engagement.

Poster #12

Richard Dortch

Diffusion MRI biomarkers of peripheral nerve trauma

Peripheral nerve damage following trauma results in catastrophic loss of sensorimotor function if not treated in a timely manner. In severe cases, surgical repair is required to regain function, but outcomes remain suboptimal (with a failure rate reaching 40%). While electrodiagnostics are valuable indicators of nerve function and muscle denervation, they are often challenging to interpret early post-injury, limiting our ability to determine if surgical intervention is warranted. After surgery, it can also take many months for electrodiagnostics to indicate whether axons are sprouting across the repair site and regenerating toward their motor or sensory target. In both cases, this often results in a "wait and watch"approach that relies on the clinical manifestations of reinnervation (e.g., the return of sensorimotor function), which ultimately delays clinical decision-making and in- creases the likelihood of permanent muscle

atrophy, sensory loss, and the formation of painful neuromas. Given these limitations, a biomarker that monitors nerve regeneration throughout the recovery process would improve sensorimotor outcomes by allowing for the earlier identification of i) nerves that require surgery and ii) failed repairs after surgery, even guiding re-operation (when necessary) in the latter case. Diffusion tensor im- aging (DTI) is an MRI method that yields indices (e.g., fractional anisotropy, FA) sensitive to nerve pathologies. We previously demonstrated that i) FA values from *ex vivo* rat nerves relate to axon density and behavioral outcomes following trauma and surgical repair and ii) FA values from human nerves report on failed surgeries, successful reoperations, and injury severity. While promising, larger-scale studies are required for clinical vali- dation given the heterogeneous nature of traumatic nerve injuries. Furthermore, we know that DTI lacks specificity in the presence of concurrent edema and de/regeneration early after trauma. In line with these challenges, our overarching goal is to move nerve diffusion biomarkers toward clinical trial readiness by i) developing advanced diffusion methods with increased pathological specificity to regeneration; ii) demonstrating consistency across MRI vendors/sites; iii) and providing clinical validation by expanding to a larger-scale, multi-site study to evaluate whether pre- and post-surgical diffusion MRI predicts clinical outcomes. This multi-PI project represents a unique collaboration between scientists with expertise in advanced peripheral nerve MRI and world-class peripheral nerve surgeons. We will use the complementary technical and clinical expertise of the team to identify novel diffusion-based biomarkers based on the spherical mean technique (SMT) and optimize/evaluate performance. We hypothesize that SMT parameters predict surgical outcomes with higher levels of sensitivity and specificity than both DTI and standard clinical methods. If successful, these SMT-based biomarkers will allow physicians to recommend surgical interventions and detect failed repairs earlier than is currently possible. Once established, these methods will also likely be of clinical utility in proximal injuries, where the prognosis for recovery is currently poor due to the prolonged time required to detect failed regeneration.

Poster #50

Julieth Gómez, Grace Lowor, Kelly D. Foote, Michael S. Okun, Aysegul Gunduz

Analysis of chronic recordings in centromedian thalamus and globus pallidus interna for closed loop deep brain stimulation in Tourette syndrome

Tourette Syndrome (TS) is a neuropsychiatric disorder characterized by repetitive and involuntary motor and phonic tics. Deep Brain Stimulation (DBS) has shown promise as a therapeutic option for refractory TS, particularly in alleviating motor tics. This study explores the identification of targets of tic detection and suppression in the Centromedian (CM) Nucleus of the Thalamus and the Anterior Globus Pallidus Interna (aGPi) using closed-loop DBS. The study spans nine months, comprising several phases: identifying the best nuclei for tic detection and suppression, optimizing adaptive settings, and testing the optimized settings. Data collection involves rest periods, voluntary movement of hands upon cue presentation, and periods of tics while recording video and streaming data from the Medtronic Percept implants and Delsys Wearable Sensors. The recorded data is aligned and marked for analysis, comparing Local Field Potential (LFP) power and time domain data recordings during different conditions. The study involves eight subjects, with one patient fully implanted at present. Our preliminary results are from a female subject with bilateral DBS electrodes targeting CM and aGPi, and bilateral Percept Neurostimulators. Our analysis reveals that lower frequency bands (1-10 Hz) show consistent separability of tics from rest for this subject (p-value < 0.1). However, due to hardware limitations, the lowest observable range for closed-loop DBS is 7.81 ± 2.5 Hz. Despite this constraint, we have successfully initiated closed-loop DBS using the LFP signal. Future directions include periodic evaluation of stimulation thresholds to maintain therapeutic efficacy, prevent subject habituation, and improve closed-loop DBS parameters optimization techniques based on real-time and chronic monitoring of

neural activity and symptom progression. Additionally, investigating the impact of CM and aGPi stimulation on TS-associated psychiatric comorbidities is crucial. While the first subject does not present with psychiatric comorbidities, future subjects will shed light on the effects of DBS on these conditions. Lastly, we aim to explore alternative approaches for efficient tic detection and marking during patient visits, as the current video labeling technique is time-consuming. In conclusion, our study demonstrates the potential of closed-loop DBS targeting CM or aGPi for TS treatment. Further research is needed to optimize DBS parameters and monitor symptom progression, ultimately leading to tailored therapies that account for individual patient characteristics.

Poster #26

Julia Bandura, Guangmin Yao, Matthew R. Parris, W. Cedric Kuo, Peter Pörzgen, Brandi Castillo, Evan S. Mason, Andres Chinchilla, Junhao Huang, Sayuri Suzuki, Rylee Ross, Ellis Akana, Savana Vander Schuit, Xi Gong, Steven Miller, Reinhold Penner, Hong-Shuo Sun, Zhong-Ping Feng, Kenneth G. Hull, Daniel Romo, Andrea Fleig, F. David Horgen

Development of novel TRPM7 inhibitors based on waixenicin A as a treatment for hypoxic ischemic brain injury

Neonatal hypoxic-ischemic brain injury (HIBI) and its resultant condition, hypoxic-ischemic encephalopathy, is a major health concern, occurring in 2% of full-term and 60% of premature infants. Therapeutic hypothermia is the first evidence-based neuroprotective therapy for HIBI but has a narrow diagnostic window and approximately half of hypothermia-treated neonates still experience adverse outcomes despite reduced mortality. Thus, it remains critical to identify novel therapeutic agents to enhance neuroprotection following HIBI. A key drug target for HIBI is TRPM7, a ubiquitously-expressed, non-selective divalent cation channel that has been shown to mediate hypoxic-ischemic brain cell death. Recently, our group has shown that inhibition of TRPM7 by waixenicin A (WaixA), a xenicane diterpenoid isolated from the Hawaiian soft coral Sarcothelia edmondsoni, reduces brain injury and improves functional outcomes following HIBI in neonatal mice. To optimize and enhance its therapeutic potential, we sought to use WaixA as the basis for identification of novel compounds for treatment of HIE. To this end, we have isolated additional congeners of WaixA and synthesized derivatives of WaixA through chemical transformations of naturally-occurring WaixA. We will describe these synthetic studies and their in vitro inhibition of TRPM7 through patch-clamp experiments. The described studies represent a significant step in the development of a potent, selective WaixA derivative with potential for clinical use in treatment of HIBI and HIE.

Poster #72

Prabesh Kanel, Alexis Griggs, Taylor Brown, Giulia Carli, Roger L. Albin, Nicolaas I Bohnen

The challenges in translational research at Michigan Udall Center

Human subjects research presents unique challenges for participants and study team members. A common administrative challenges is the cost associated with human subject research, which can be a significant barrier to conducting studies. Recruitment and retention of cohorts is also a notable challenge, especially in rare or debilitating diseases where participants may have to commit multiple days to complete studies. In older patient populations, it can also be complicated to schedule participants for study visits due to scheduling conflicts, variable driving capabilities, and/or caregiver availability. Compliance with interventions, such as devices or difficult-to-track drugs can interfere with interpretation of clinical research data. Study teams may have to decide whether the participant can continue the study based on tolerability and compliance with the study drug or device. There is always a concern for participant comfort and safety, especially in our older patient population. Study team

members do their best to reduce the risk of harm in as many ways as possible, which may result in skipped assessments that are vital to research studies. The current Udall Center project, a prospective cohort study, encountered problems with longitudinal assessment of prior funding cycle study participants during a renewal approval period when no bridge funding was available. With successful renewal of the Center, we re-contacted prior funding cycle participants, but as is the case when following up older subjects with neurodegenerative disorders, there was significant attrition due to the underlying illness and other age-associated medical comorbidities. Lapse in funding compromises the initial investment in uniquely characterized subject cohorts. We suggest consideration of a mechanism for quickly approved bridge or modest continuation funding to maintain minimum clinical level assessments to prevent the loss of precious participants, especially those who are frail and at more advanced disease stages.

Poster #48

Catherine Demos, Daniel Romero, Nikhil Padmanabhan, Rachel Cohen, Jermaine Brown, Christopher Campbell, Martin Stengelin, Anu Mathew, George Sigal, Jacob Wohlstadter
Analytical validation of an ultrasensitive neuroinjury multiplex assay panel

Three blood-based biomarkers of neuroinjury—glial fibrillary acidic protein (GFAP), neurofilament light (Nf-L), and total Tau (tTau)—have emerged as promising biomarkers for research regarding neurological disorders and neuroinjuries such as hypoxic-ischemic encephalopathy (HIE), traumatic brain injury (TBI) and Alzheimer's disease (AD). The low levels of GFAP, Nf-L, and tTau in serum and plasma require highly sensitive assays to detect these important biomarkers. Here, we report on an ultrasensitive, electrochemiluminescence-based, multiplexed immunoassay for GFAP, Nf-L, and tTau that has been analytically validated, providing a new tool for assessing neuroinjury biomarker research. The MSD S-PLEX Neurology Panel 1 kit uses ultrasensitive S-PLEX assay technology to simultaneously measure GFAP, Nf-L, and tTau in a 96-well plate format using standard liquid-handling techniques. Analytical validation was carried out in a series of studies based in part on Clinical and Laboratory Standards Institute guidelines EP05-A3, EP06-Ed2, EP07-Ed3, EP17-A2, EP25-A, and EP28-A3c to evaluate precision, dilution linearity, interference screening, detection capability, stability, spike recovery, and cross reactivity. The assay requires 5 µL of a sample for each replicate. The time to result is less than 6 hours and the quantifiable range of the assay is 2.4–4,230 pg/mL for GFAP, 7.65–10,000 pg/mL for Nf-L, and 0.34–735 pg/mL for tTau. A set of 15 common potentially interfering substances were screened in serum and plasma, and none showed interference exceeding 18% difference in measurement compared to non-spiked samples. Within-laboratory precision was <11% CV for GFAP and Nf-L and <19% CV for tTau for samples spanning the reportable range. Dilution up to 256-fold recovered within 80-120% of the expected value. MSD has developed and analytically validated a highly sensitive, multiplexed immunoassay for quantifying GFAP, NfL, and tTau in plasma and serum. This assay is sufficiently sensitive to detect these analytes in normal serum and plasma and has a large dynamic range to accommodate elevated levels found in some neurological disorders.

Poster #71

Pamela Kell, Sonali Mishra, Elena-Raluca Nicoli, Precilla D'Souza, Cynthia J Tifft, Xuntian Jiang
Application of a pentasaccharide biomarker to access treatment efficacy of gene therapy for GM1 gangliosidosis

GM1 gangliosidosis is a rare and lethal neurodegenerative disorder caused by mutations in the *GLB1* gene. These mutations lead to a deficiency in β-galactosidase enzyme activity, resulting in the accumulation of glycoconjugates with a terminal β-galactose. Promising advancements have been made

in the treatment of this condition using adeno-associated viral (AAV) gene therapy. In a cat model of GM1 gangliosidosis, this therapy demonstrated the ability to delay the onset of symptoms, reduce storage in the brain and peripheral tissues, and increase lifespan. These favorable outcomes have laid the foundation for early-stage AAV gene therapy trials. Given the slow progression of the disorder, particularly in late-infantile and juvenile forms, the availability of validated biomarkers would significantly enhance the assessment of therapeutic efficacy in AAV gene therapy. Recently, we have successfully identified and synthesized a pentasaccharide biomarker called H3N2b, which exhibited an elevation of over 18-fold in patient plasma, cerebrospinal fluid (CSF), and urine. Importantly, H3N2b is a natural substrate of β-galactosidase. To ensure accurate quantification of this biomarker in biospecimens, we have developed fully validated assays. In a Phase 1/2 intravenous AAV9 gene therapy trial for GM1 gangliosidosis, we observed a significant reduction of H3N2b levels in patient urine, plasma, and CSF samples. This reduction correlated with an increase in β-galactosidase activity. These findings strongly indicate that the pentasaccharide H3N2b can serve as a valuable pharmacodynamic biomarker for monitoring therapeutic response. Moreover, its implementation may have far-reaching implications for expediting drug approval processes in this rare genetic disease.

Small Business Program (SBIR & STTR)

Poster #47

Laurie Lambot
Pioneering neuroscience advancements: Modendo's ultra-thin probes for therapeutic insights
Modendo is leading the development of endomicroscopes equipped with ultra-thin, minimally-invasive probes. This cutting-edge instrumentation addresses a critical need within the neuroscience community, advancing our understanding of the brain. Our technology enables researchers to explore and visualize brain activity in previously challenging regions, such as the brainstem and olfactory bulb, with minimal impact on neural structures.

The ability to insert multiple imaging probes into intricate brain areas offers a unique opportunity to expand our knowledge of the brain's connectome. By correlating neural stimulation and activity across various brain regions, we aim to uncover the complex network of connections governing brain function. This advancement promises valuable insights into the functional disparities between healthy and diseased brains, potentially leading to improved treatments and interventions.

Modendo's innovation extends beyond imaging. Its comprehensive system solution enables projection of precise laser patterns for both photo-stimulation and selective energy delivery. This versatile approach opens opportunities for a wide range of applications, from accurately targeting and modulating neural activity to the selective removal of problematic tissues.

We invite you to engage in discussions with us about the diverse applications of our ultra-thin probe endomicroscope.

Poster #70

J. Pilotte, S. Muller, J. Molina, Z. Mandel, S. Sharma, A. Tafreshi, J.H. Kordower, S. Sarraf.
Investigating the potential utility of a retinal tracer for alpha-synuclein as a biomarker of multiple system atrophy in a non-human primate model

Introduction: Multiple System Atrophy (MSA) is a progressive neurodegenerative disorder characterized by the accumulation of α-synuclein (α-syn) aggregates within oligodendrocytes in the central nervous system. Accurate and early diagnosis of MSA remains a clinical challenge. Molecular biomarkers of several neurodegenerative diseases form in the retina, an extension of the CNS, as they do in brain. Deposits of α-syn have been observed in postmortem retina from Parkinson's patients, raising the possibility that α-syn pathology associated with MSA may also appear in retina. If so, the distribution of oligodendrocytes within the eye may provide a diagnostic signature unique to MSA. This study aimed to investigate whether a clinical-stage fluorescent retinal tracer that labels α-syn in retina using standard retinal cameras has the potential to detect α-syn as a biomarker for MSA.

Methods: MSA-like pathology was induced in a non-human primate (NHP) model by injection of an α-syn transgene expressing vector in the putamen and lateral geniculate nucleus (LGN). The retinal tracer was administered monthly via bolus intravenous (IV) injection, and its safety and tolerability were monitored over a 4-month period. To assess a-syn labeling, *in vivo* retinal images were collected as fundus fluorescence (FF) and OCT retinal images prior and post-injection using a Spectralis® retinal imaging camera.

Results: The retinal tracer exhibited rapid uptake in the retina, with visible presence in the vasculature approximately 2 minutes post-injection, followed by clearance from the retinal vasculature within 5-10 minutes. Hyperfluorescent spots observed at baseline did not increase post-injection, and no structural abnormalities were noted. Immunohistochemistry (IHC) analysis of brain sections for phospho-α-synuclein confirmed MSA pathology near the injection site. However, no phospho-α-synuclein was detected in the retina by IHC, indicating the absence of a-syn transgene expression in this tissue. Pharmacokinetic analysis of plasma samples collected at 10- and 35-minutes post-injection revealed rapid clearance of the retinal tracer.

Conclusions: Our findings demonstrate that our retinal tracer can effectively reach the retina following IV administration and be visualized using a retinal imaging device that is routinely used by eyecare providers. Furthermore, this longitudinal study highlights the well-tolerated nature of the retinal tracer, with a very short plasma half-life, even at doses multi-fold higher than the proposed maximum dose for humans. These data corroborate the pharmacokinetic and safety profile of the retinal tracer and warrant further investigation in clinical settings where retinal a-syn pathology may be present in MSA patients.

Poster #46

Camilo Sanchez, Andrew Sloan, Derrick Yoo, Aditya Ramamurthy, Jason Carmel
SensiTrak: A modular rodent testing device for forelimb somatosensory assessment

Current common methods for measuring somatosensory function in preclinical rodent models generally rely on withdrawal responses to uncomfortable or painful stimuli. These tests can be stressful to the animal, while also yielding a high variability in measured responses, and repetitive testing in longitudinal models may even result in chronic pain states. To better understand the connection between physiology and perception of touch and proprioception, researchers need a modern, off-the-shelf assessment system to administer and quantify trained, volitional behaviors. In this project, Vulintus, Inc. - in collaboration with the Carmel Laboratory at Columbia University and Hollis Laboratory at Winifred Masterson Burke Medical Research Institute - are developing and validating 'SensiTrak,' an automated, high-throughput behavioral system designed to finely measure somatosensory function in rodent models.

SensiTrak meets the needs of somatosensory-focused researchers by supporting complex, electromechanical feedback interactions with animals, with interchangeable task modules, behavioral peripherals, and open-source control software. Our system is capable of assessing tactile, vibration, and proprioception somatosensory discrimination thresholds via Go/No-Go and Two Alternative Forced Choice (2AFC) behavior tasks within a few weeks of habituation and operant training. Phase I focused on the development of a vibration detection task, with rats trained to detect vibration trains of varying duration applied to a grasping handle stimulating their forepaw. Task sensitivity was then validated by two protocols, chemical inactivation of wrist mechanoreceptors and dorsal column lesion, showing significant detection deficits on both. SensiTrak is also able to detect discrimination threshold recovery following both perturbations. Recent, ongoing Phase II experiments are developing 2AFC tests for proprioception and texture discrimination which show similar psychometric characterization.

Vulintus is eager to demonstrate the feasibility of SensiTrak's ability to assess somatosensation in a manner which more closely emulates clinical tests while concurrently reducing researchers' workload, increasing data acquisition, reducing subjects' handling-induced stress and thus increasing consistency in data quality. However, we also discuss the challenges inherent in creating new behavior training algorithms in parallel with hardware and software development.

Poster #45

Mark W. Nowak, Leigh Korbel, Lars Nilsson, Brian K. Panama Alex Kane, Michael L. Hines, Nicholas T. Carnevale, Glenna C.L. Bett, Randall L. Rasmusson

Combining dynamic clamp with NEURON simulation software: assessing effects of cloned ion currents and drug block on in silico action potential behavior

The pharmaceutical industry has devoted considerable effort to developing drugs targeting ion channels (e.g., Na channels) for treatment of neurological disorders (e.g., epilepsy, neuropathic pain) and developing screens to de-risk drug cardiotoxicity resulting from off-target interactions (e.g., Kv11.1 or HERG which exist in both neurons and cardiac tissue). We have developed the ability to assess the effects of cloned ion channel currents and drug block on neuronal and cardiac action potential (AP) behavior by interfacing our dynamic clamp system with the NEURON simulation software package (www.neuron.yale/edu/neuron/), the leading in silico system for building and testing computational cell models. This interface allows for cloned ion channel currents expressed in HEK cells to be input into in silico neurons and cardiomyocytes to examine the functional consequences of drug-channel interactions and ion channel mutations.

We used complex in silico NEURON models for a Purkinje neuron (Akemann and Knopfel, 2006) and an atrial cardiomyocyte (Courtemanche et. al., 1998; Jacobson, 1998). In silico neurons or cardiomyocytes were used to voltage clamp HEK cells expressing cloned Kv11.1. The Kv11.1 current was fed back into the in silico neuron or cardiomyocyte where the effects of Kv11.1 current and drug block were measured.

For the in silico Purkinje neuron, addition of cloned Kv11.1 current significantly decreased the number of spontaneously firing APs (Control: 15±1, n=15; 18 pA Kv11.1 current: 2±1, n=10, $p < 0.05$) in a linear manner (slope=-0.83 AP #/pA, r2=0.99).

For in silico atrial cardiomyocytes, the AP displays a spike and dome morphology with a resting membrane potential of -81.5±0.3 mV, an amplitude of 108±2 mV, a dV/dt of 209±10 V/s and an APD90 of 283±7 ms (n=17). Replacing the in silico cardiomyocyte IKr with cloned Kv11.1 resulted in similar AP morphology with a resting membrane potential of 78.4±1.5 mV, an amplitude of 106±4 mV, a dV/dt of 219±12 V/s and an APD90 of 277±6 ms (n=7). Dofetilide block (30 nM) of the cloned Kv11.1 current resulted in significant prolongation of the APD90 (0 nM Dofetilide: 255±12 ms vs. 30 nM Dofetilide: 337±12 ms, $p<0.05$, n=5).

We used an NaV1.7 Markov model to study the effects of state-dependent drug binding on Na channel activation and AP behavior in the Purkinje in silico neuron. Drug binding to the I1 fully inactivated state resulted in block of NaV1.7 peak current with an IC50 value of 59.1±7.3 nM. In contrast, drug binding to I1 displayed a higher potency on AP inhibiting firing with an IC_{50} value of 8.3±0.5 nM. These results show a disparity between drug block under voltage-clamp and the inhibitory effects on neuronal excitability.

Combining NEURON simulations with a real-time dynamic clamp system allows for assays targeting ion channels on a standardized in silico predictor of AP behavior. Evaluating mutations and drug therapy can be made patient-specific using cloned channels containing the pathological mutation with this approach.
These studies were supported, in part, by NIH NS011613 and 1R43NS125749.

Poster #3

Tishan Williams, Christian Kinney, Charles Marusak, Kevin Kim, Fu-An Kang, Anthony Saleh
Development of MC-DX4 as an antibody-ASO conjugate therapeutic for facioscapulohumeral muscular dystrophy (FSHD)

FSHD, the third most common inherited muscle disorder, is characterized by progressive weakening and wasting of skeletal muscles due to the aberrant expression of the *DUX4* transcription factor. While *DUX4* is not directly "druggable" by small molecules several studies have demonstrated the utility of antisense oligonucleotides (ASO) in repressing *DUX4* expression levels, thereby reversing muscle pathology in pre-clinical models. We have applied our patented DREAmiR™ platform to accurately assess *DUX4* gene sequence conservation and expression patterns in FSHD patients to identify optimal target sequences for therapeutic ASOs. Additionally, we qualify the structure, affinity and potential toxicities of our designed ASOs to ensure miRecule develops a best-in-class therapeutic ASO that can repress DUX4 expression while co-targeting pathways involved in FSHD pathology without eliciting an inflammatory response. A significant hurdle for the development of ASOs as therapeutics for muscular dystrophies is the ability to deliver effective doses into affected muscle cells. To address this issue, we analyzed the protein expression profiles of hundreds of patients covering 20 different neuromuscular disorders to identify potential targets that will allow specific delivery of our ASO to skeletal muscle cells. We identified five high-priority surface receptors with stable expression despite disease progression, that are lowly expressed in non-muscle tissues, and highly expressed on skeletal muscles. Using our NAVIgGATOR™ technology, miRecule is developing an antibody-ASO conjugate therapeutic (MC-DX4) that specifically delivers our DUX4-targeting ASO to skeletal muscles for the treatment of FSHD.

Poster #68

Yoonbae Oh, Hojin Shin, Abhinav Goyal, Juan Rojas-Caberera, Kristen Scheitler, Graham Cameron, Jason Yuen, Warren O. Dennis, Diane R. Eaker, Joshua B. Boesche, Ian Mandybur, Basel Sharaf, Dong-Pyo Jang, Charles D. Blaha, Kevin E. Bennet, Kendall H. Lee
A development of a device for simultaneous fast scan cyclic voltammetry, multi-cyclic square wave voltammetry, electrophysiology, and stimulation

Background: There has been significant progress in understanding the role of neurotransmitters and their release in the context of normal and pathologic brain function. However, little is known about real-time *in vivo* neurochemical changes as a function of dynamic brain processes such as disease progression and response to pharmacologic, cognitive, behavioral, and neuromodulation therapies. This is due at least in part to a lack of research tools capable of measuring these dynamic changes in brain activity *in vivo*.

Method: Here, we present a research platform, WINCS MAVEN developed by Mayo Clinic Neural Engineering Laboratories and Division of Engineering, which can measure with four independent sensor channels and four independent stimulation channels and characterize real-time *in vivo* changes in neurochemical and electrophysiological activity across multiple anatomical targets to study normal and pathologic brain function

Results: Main acquisition, Field Programmable Gate Arrays and stimulation circuits are being developed for multi-modal recordings and electrical stimulation. The battery-powered MAVEN device communicates with the base station using optical cable for MCSWV and via Bluetooth for all other functionalities. We demonstrate several key features of the MAVEN system in different functionalities. These features include measurement and characterization of neurochemical signals, real-time synchronization with therapeutic interventions such as electrical stimulation enabling users to act on

these changes to provide real-time feedback to control neurochemical levels and aid in optimizing therapeutic efficacy.

Conclusion: The MAVEN system described here will improve understanding of the dynamics of brain physiology in the context of neurologic disease and therapeutic interventions, which may lead to the development of precision medicine and personalized therapies for optimal therapeutic efficacy.

Poster #67

Kathryn Ozgun, Adam J. Dixon, Zachary Leonard, Paul S. Sheeran, Delphine Le Roux, Sylvia Caldwell, F. William Mauldin, Jr.

Development of an ultrasound-based spinal navigation system for interventional procedure guidance and administration of therapeutics

Introduction: Advances in interventional procedure guidance technologies are associated with reduced mortality, accelerated recovery, and reduced healthcare costs. Ultrasound (US) guidance has been shown to improve success rates for applications in pain management and therapeutic administration. However, widespread utilization of US-based procedure guidance has been limited by the learning curve required to gain proficiency, challenging workflow involved with manipulating an imaging device during needle insertion, and image interpretation in challenging body habitus. Aided by NINDS SBIR Phase II funding, Rivanna Medical has developed the Accuro 3S, a highly automated spinal navigation system for interventional procedure guidance in bony anatomy. The Accuro 3S uses AI-based anatomical recognition and proprietary BoneEnhance™ image processing technology to aid visualization of bone landmarks involved in procedures such as lumbar puncture and epidural/intrathecal administration. Beyond these imaging advances, a novel dual-array format allows a 'through the probe' needle technique that simplifies needle trajectory planning. To develop a feature set that addresses common obstacles in US-guided interventional procedures, formative research and usability studies were conducted through the design input and design process phases of product development.

Methods: To acquire feedback that would inform development, we conducted semi-structured interviews with more than 30 providers with expertise spanning anesthesiology, neurology, hematology/oncology, and chronic pain management. All interviews were conducted privately. Participants were shown conceptual depictions of the Accuro 3S product and asked to score their level of agreement on the perceived value of select features and their impact on clinical outcomes on a scale of 1 to 5, with 5 being the most impactful. These market research results were used to guide product prototyping in 10 formative and summative usability studies.

Results: In the market research phase, the two features that ranked highest were development of a 'hands-free' imaging capability (average score of 4.41) and inclusion of a single-use sterile kit that simplifies workflow (4.21). Participants perceived the presence of AI automation as helpful (4.14) and anticipated the conceptual design of the sterile kits would be easy to use (4.0). When asked about the impact on clinical outcomes, the Accuro 3S was perceived as helpful for reducing the number of needle insertion attempts (4.03) and for increasing patient safety (3.97). Respondents were neutral or slightly positive as to whether US-guidance will become the standard of care long-term (3.28). Iterative usability studies refined feature design to produce a compact, cart-based platform that satisfied user needs related to hands-free operation, sterile kit functionality, and needle guidance imaging capabilities.

Conclusion: The Accuro 3S feature set was well received in market research results and usability studies. Participants believed the device would simplify workflow, reduce training requirements, and would have a high likelihood of decreasing the number of attempts needed to perform interventional procedures.

Aided by NINDS SBIR funding, the Accuro 3S design process is near completion and clinical evaluation is planned for early 2024 in applications of epidural injection and lumbar puncture. Continuing research will explore expansion into applications of regional anesthesia, chronic pain management, and intrathecal administration of therapeutics.

Poster #4

Michelle K. Mattson-Hoss, Mya Walker, Gwen Guzman, Michael Frost, Glenn Croston, Herb Sarnoff
Haploinsufficiency correction therapy (HCT): Disease modifying therapeutic discovery for neurofibromatosis type 1

iNFixion Bioscience is a startup in San Diego, CA, working to discover and develop therapeutics for Neurofibromatosis Type 1 (NF1). NF1 is a rare, haploinsufficient genetic disorder occurring in 1 of 3,000 live births and impacting an estimated 120,000 individuals in the US and 2.5M worldwide. Symptoms vary widely and include nervous system tumors (plexiform neurofibromas) that can transition into malignant sarcomas in 8-13% of patients, cutaneous neurofibromas (skin bumps) that can number into the thousands, scoliosis, pain, itch, sleep complications, cardiovascular abnormalities, increased cancer risk, and learning and social deficits that can greatly impact quality of life. Available treatments are limited to individual symptom management, but only if a therapy is available. Consequently, there remains an immense need to develop therapies to address the wide breadth of symptoms experienced by patients with NF1. At iNFixion, our approach is to develop therapies that address the underlying cause of NF1 - insufficient neurofibromin protein. By targeting the mechanisms that regulate the production and degradation of neurofibromin, we aim to increase the amount of functional protein, thus addressing the multiple symptoms in NF1 that result from haploinsufficiency. With a focus on accelerated clinical impact, our lead programs focus on identifying small molecule known drugs that can be repurposed to increase neurofibromin protein expression or stability. Multiple compound classes have been identified that increase neurofibromin protein levels and are being evaluated for progression to drug leads that safely increase neurofibromin protein levels and correct altered neurofibromin-dependent signaling pathways. An additional discovery approach being pursue by iNFixion Bioscience is the identification of stericblocking oligonucleotides (SBOs) that specifically block miRNA-mediated downregulation of NF1 mRNA. We have identified several highly expressed miRNAs that regulate NF1 mRNA, and are designing chemically modified SBOs to block these binding sites. This putatively will result in an increase in neurofibromin protein and an impact on dependent signaling pathways. In parallel to our therapeutic programs, we are striving to develop preclinical and clinical methods that successfully translate efficacy. As such, we are developing and qualifying new preclinical models for testing our lead programs *in vivo*. Additionally, we are in the process of NF1 blood-based biomarker discovery to ultimately create a pharmacodynamic biomarker that will guide the dosing of our NF1 haploinsufficiency-correction therapeutics. Success in developing improved preclinical models and biomarkers will benefit our programs as well as others who are developing therapies targeting the underlying cause of NF1. Our primary goal is to fundamentally improve the lives of NF1 individuals, regardless of their specific NF1 genetic mutation. To this end, we are developing different therapeutic modalities to correct the driver of a broad range of NF1 symptoms and to build new preclinical/clinical tests to improve translatability for our programs.

Poster #5

Ana C. Puhl, Sarah Negri, Maggie Hupcey, Sean Ekins
Developing treatments for rare diseases on a shoestring: the Batten disease (CLN1) enzyme replacement therapy experience

One of the many challenges for working on a rare or ultra-rare disease is the availability of funding. Our approach at Collaborations Pharmaceuticals, Inc. has not required Venture Capital, Angel investment or funding by Foundations so far. We have instead pursued NIH small business grants to fund the early preclinical work performed by our academic collaborators and ourselves. In 2019 we were awarded a Phase I SBIR from NINDS to fund the development of an enzyme replacement for an ultra-rare disease, Batten disease CLN1 and to perform efficacy assessment in a knock-out mouse model. In parallel, we have also applied our in-house machine learning approaches and *in vitro* methods to identify inhibitors and potential chaperones for the enzyme involved, PPT1. We also participated in the NIH I-Corps program (Spring 2019 cohort) to perform customer discovery and plan the commercialization pathway. We conducted 164 interviews with various stakeholders and learnt about our product development and pathway moving forward to lead to the customer. This preliminary work was funded for around $300,000. This initial work was then used to write a larger Phase II SBIR grant and led to the recent $3M award which will enable us to develop GLP quality recombinant PPT1 protein, perform an IND enabling 3-month dog toxicity study as well as submit a pre-IND to the FDA. We will describe the progress of this project, our plans for the further development of this project, and the pros and cons of our strategy. To date, we have evaluated 10 CDMOs and selected one to go forward with, selected a regulatory consultant, initiated our quality documentation and had our pre-IND meeting. We will provide a “how to” plan so that others may be able to follow us in the future as our insights may be more broadly applicable to other rare diseases and inspire others to take a similar route. We believe no disease is too small and our shoestring approach could drive more interest in developing such treatments.

Translational Devices

Poster #74

He Zheng, Karim Oweiss
Optimizing deep brain stimulation for treating refractory severe essential tremor

Deep-brain stimulation (DBS) has been an effective treatment for movement and cognitive disorders, particularly for Parkinson's disease (PD) and essential tremor (ET). However, a significant PD and ET patient subpopulation develop significant side effects and become refractory to DBS over time, requiring clinicians to continue to manually adjust the total electric energy delivered (TEED) within the maximum tolerated level by the patient. Although the mechanisms by which decline in DBS efficacy over time remain unclear, minimizing TEED while maximizing tremor suppression may help prolong the longevity of DBS efficacy. However, there are currently no systematic strategies for optimizing DBS parameters to elongate its efficacy.

Here we propose an Artificial Intelligence (AI)-based approach to systematically optimize DBS parameters that minimize the TEED while maximizing tremor suppression. As tremor and the response to DBS therapy likely depend on patient physiology, the type of motor task, and the longitudinal time point in treatment, we hypothesized that these dependencies could be extracted from local field potentials (LFP) features that are concurrently recorded during DBS therapy. The approach is comprised of three basic models interconnected in a feedback control framework. Model 1 seeks to predict the current tremor—in terms of limb accelerometry (ACC) - given LFP features and current DBS parameters. Model 2 seeks to predict new DBS parameters using LFP features and the measured tremor. Model 3 seeks to adjust these DBS parameters using the error between the measured and desired level of tremor suppression.

We tested this framework in human patients implanted with dual DBS leads in the thalamic ventralis intermediate (VIM) and ventral-oralis complex (VO) nuclei using models consisting of Recurrent Neural Networks (RNNs). We first found that clinician DBS programing results in substantially lower tremor over the first 12-18 months of follow-up visits. We then found that RNN model fitting of tremor and DBS TEED relationship substantially outperforms linear regression. We then found that LFP features generally improve model accuracy, suggesting that DBS programing can be substantially improved using neural biomarkers of tremor and/or task-related motor behavior. Our results suggest that this novel framework provides a systematic way to narrow down the space of DBS parameters that cannot be all tested in clinic due to time constraints and provides further guidance to clinicians to potentially slow down or mitigate the risk of DBS efficacy decline.

Poster #11

James Abbas, Sathyakumar S. Kuntaegowdanahalli, Anil K. Thota, Andres E. Pena, Kenneth W. Horch, Aliyah Shell, Tommaso Benigni, Diego Aguilar, Justin Asbee, Erika Petersen, Mark Tait, John Bracey, and Ranu Jung
Enhancing sensorimotor integration using a neural enabled prosthetic hand system

There is a large and growing population of people with upper limb amputation whose needs are not being fully met by current prosthetic hand technology. The long-term goal of the proposed work is based on the notion that prosthetic hand technology that can provide task-related sensations to a person with amputation will increase their proficiency in performance of sensorimotor tasks and will allow them to participate in a greater range of employment and leisure activities.

The Adaptive Neural Systems neural-enabled prosthetic hand (ANS-NEPH) system was designed and developed by our group in collaboration with industrial and clinical partners to provide prosthesis users with task-relevant sensations. The system uses signals derived from sensors on an instrumented prosthetic hand to elicit sensations by delivering stimulation via fine-wire longitudinal intrafascicular electrodes implanted in peripheral nerves of the residual limb. Importantly, this type of stimulation elicits sensations that are referred to as the phantom hand. The system is suitable for daily use in real-world environments; all components are either implanted or prosthesis-mounted. It uses wireless communication to enable prosthesis-mounted electronics to control stimulation delivered by the implanted neural stimulator. Donning, doffing, and operation of the system is comparable to what is required to use a commercially-available myoelectric prostheses.

Under an investigational device exemption (IDE) from the US Food and Drug Administration (FDA), we are currently conducting a first-in-human clinical trial of the ANS-NEPH system. Each participant will have the implanted components surgically installed, be fitted with the external components of the system, participate in an extensive series of experiments designed to assess long-term viability of our approach, and use it on a daily basis as their primary prosthetic hand. The clinical trial is designed to gather data from each participant periodically over the first two-years following implantation of the neurostimulator.

The primary outcome of this work will be a demonstration of clinical feasibility of a neural-enabled prosthetic hand system for daily use at home or at the workplace that uses wirelessly-controlled implantable stimulation technology. For people with transradial amputation, real-time task-related sensation is likely to improve sensorimotor capabilities and everyday use is likely to enhance embodiment of the prosthesis by the user. Furthermore, stimulation of afferent fibers may also reduce the severity and incidence of phantom limb pain. This system forms the foundation for systems to be developed that can provide sensation to people with other types of upper limb amputation (transhumeral, shoulder disarticulation) and people with lower limb amputation. Finally, the deployment and chronic use of an implantable system that enables stimulation of discrete sets of small groups of afferent fibers may pave the way for future uses of this technology to activate peripheral nerves that may influence metabolic processes, enhance immune system function, regulate gastrointestinal activity, or treat a variety of medical conditions.

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Poster #69

Ryan Shores, Tessy Thomas, Amada Abrego, Jace Willis, Nitin Tandon, John Seymour
DIrectional and SCalable (DISC) microelectrode array for speech decoding

Currently, the brain-computer interface (BCI) field has demonstrated two distinct device strategies - macro-electrodes (e.g., surface grids and depth) versus microelectrode arrays, and some are even pushing the field to smaller, higher density arrays hoping to address the general signal degradation. Both approaches have been in development for decades. However, BCI devices to treat aphasia, dysarthria, or locked-in syndrome also need to access deeper brain regions given the very large, parallel networks involved in speech. Consider that two-thirds of the cortex is buried beyond the reach of most state-of-the-art technologies.

We have designed a novel approach to brain recordings to address the challenge of multi-scale recordings at any desired depth. Our team presents a novel device whose form is based on the proven

safety and utility of the stereo-EEG (SEEG). We created a directional and scalable local field potential array (DISC) using the phenomenon of "substrate shielding". This is not the first combination micro/macro device but is the first to demonstrate stereo-local field potentials using a patent pending design. Our preliminary *in vivo* data demonstrates significant improvement when using DISC in many critical factors predictive of future BCI performance: (i) signal amplitude, (ii) signal-to-noise ratio, and (iii) source separation in classification tasks. This project will allow us to safely test word decoding performance both offline and online in epilepsy volunteers from speech regions.

We are developing a robust DISC hybrid assembly with 128 recording channels per implant. Each implanted device will be a commercially available SEEG combined with microelectrodes without any modification to the clinical function of the device. Aim 1 will include verification, validation, biocompatibility, and electrical safety testing. Aim 1 will also include functional and safety studies in animals to complete our effort to provide a safe, reliable system prior to human feasibility studies.

Upon receiving an FDA investigational device exemption, this novel recording system will demonstrate the effect size and variance of word and speech decoding in humans as compared with conventional ring electrodes. Typically, 12-20 depth arrays are used in epileptogenic monitoring, and we will replace several of these SEEGs with a DISC hybrid assembly in 8 experimental patients and compare decoding performance to the within-patient controls and with a separate 8 patients having SEEG electrodes only. Enrolled volunteers will conduct overt and covert speech tasks. Positive results will inform and enable a word and speech decoder for persons suffering from locked-in syndrome and eventually non-fluent aphasia.

We are currently in year 1 of device development and will present our novel system design and progress toward an IDE application. Furthermore, we will present our progress in speech decoding with conventional SEEGs.

Poster #54

Kristin K. Sellers, Daniela A. Astudillo Maya, Joline M. Fan, Catherine Henderson, Joshua L. Cohen, Ghassan S. Makhoul, Kai Woodworth, Ankit N. Khambhati, Hashem Zamanian, A. Moses Lee, Rebecca Martinez, Clara K. Starkweather, Inhauck Choi, Joncarmen Mergenthaler, Elissa Hamlat, Leo P. Sugrue, Vikram R. Rao, Heather E. Dawes, Katherine W. Scangos, Edward F. Chang, Andrew D. Krystal
Identification of personalized therapeutic deep brain stimulation targets for major depressive disorder

Deep brain stimulation (DBS) is a form of neuromodulation in which electrical current is delivered to targeted areas of the brain with the goal of modulating activity. DBS for the treatment of MDD has been very successful in some applications[1,2], but has also failed to replicate in larger studies[3,4]. The Presidio clinical trial (NCT04004169) includes two novel approaches: (a) personalized stimulation target identification across multiple brain regions, and (b) closed-loop stimulation based on a symptom biomarker.

We present results of personalized stimulation testing for the first set of participants. Stimulation was tested across implanted brain regions, at multiple amplitudes and frequencies, and for varying durations. Therapeutic stimulation targets were identified for each participant: right ventral capsule / ventral striatum (VC/VS) for Participant 1[5,6]; right orbitofrontal cortex and right subgenual cingulate (SGC) for Participant 2; right SGC and left nucleus accumbens for Participant 3; and left VC/VS and bed nucleus of the stria terminalis for Participant 4. The identification of different optimal stimulation sites among the bilateral targets may reflect underlying heterogeneity in disease circuitry. We present our

stimulation testing procedures, evidence for therapeutic benefit at the identified sites, and challenges encountered in the identification of therapeutic stimulation parameters.

We also discuss our observations and learning to date regarding our clinical trial format, which includes a double-blind randomization period to assess symptoms during biomarker-driven closed-loop stimulation, intermittent stimulation, and sham stimulation.

Poster #53

Humza N. Zubair, Mauricio V. Martelo, Julia Schneiders, Jay Gill, Anthony Jang, Rahman Mustapha, Jonny Baham, Ralph J. Koek, Martin Seeber, Matthias Stangl, Sonja Hiller, Uros Topalovic, Cory Inman, Ausaf Bari, Jonathan Kao, Avishek Adhikari, Michael Fanselow, Michelle Craske, Scott Krahl, James W.Y. Chen, Merit Vick, Nick Hasulak, Nanthia Suthana, Jean-Philippe Langevin
Responsive neurostimulation for post-traumatic stress disorder: interval update

Background: Treatment-resistant Post-traumatic stress disorder (tr-PTSD) is marked by persistent basolateral amygdala (BLA) reactivity to trauma reminders. We are investigating the use of Responsive Neurostimulation (RNS, Neuropace) to modulate BLA activity in patients suffering from tr-PTSD. This closed-loop device allows for the detection of specific electrophysiological (EP) signals and for modulation of neuronal activity using on-demand stimulation.

Method: Three tr-PTSD patients have undergone the placement of the RNS. For each patient, we collect EP data during symptom-provoking laboratory tasks and during real-life events. We program the RNS to detect EP activity associated with PTSD symptoms and we are stimulating in response to the detections.

Results: We found that an increase in BLA theta power was associated with symptomatic and aversive phases of PTSD. The theta power increase was seen across different tasks including the international affective picture system and an exposure session. The first participant is currently in clinical remission with a score of 8 on the Clinician-Administered PTSD Scale for DSM-5 (CAPS-5). The second participant has experienced a meaningful clinical improvement but continues to require changes in programming algorithm. Both patients demonstrated a reduction in theta activity over time as the symptoms improved. In addition, daily theta activity fluctuations matching symptomatic phases reversed in the responder with a reduction during daytime. The third participant has not yet experienced a meaningful improvement.

Conclusion: BLA activity in the theta range may be associated with an enhanced fear state. When providing on-demand stimulation in response to theta power increases, we achieved a meaningful clinical response in two subjects thus far. We continue to adjust the stimulation parameters and our efforts towards future subject enrollment for a target of six participants.

Poster #52

Nicole R. Provenza, Vigi Katlowitz, Garrett P. Banks, Nisha Giridharan, Sameer Rajesh, Nabeel Diab, Gabriel Reyes, Sandesh Reddy, Anthony Allam, Saurabh Hinduja, Michelle Avendano-Ortega, Sarah McKay, Ben Shofty, Andrew D. Wiese, Jeffrey A. Herron, Eric A. Storch, Jeffrey F. Cohn, David A. Borton, Wayne K. Goodman, Sameer A. Sheth
Identification of candidate neural biomarkers of obsessive-compulsive symptom intensity and response to deep brain stimulation

Despite the success of deep brain stimulation (DBS) for treatment of refractory obsessive-compulsive disorder (OCD), there are currently no robust neural signatures for obsessive-compulsive (OC) symptoms or initial mood and energy improvements associated with DBS. This may be due to limited opportunities

available for conducting intracranial electrophysiological recordings during natural symptom fluctuations. Recently available DBS platforms offer a way over this hurdle, allowing for streaming of intracranial local field potentials (LFP) at home and in the clinic. Here, our goal was to identify neural correlates of OC symptom intensity and acute changes in mood and energy. We conducted longitudinal intracranial recordings in ten participants with refractory OCD implanted with recording-capable DBS devices targeted to ventral capsule/ventral striatum (VC/VS). Five of ten participants were implanted with additional sensing electrodes placed over the orbitofrontal cortex (OFC). We captured LFP at home during naturalistic exposures to OCD triggers, and in the clinic during variations in stimulation amplitude. All five participants who completed the study were clinical responders to DBS therapy. Using the intracranial data collected during OCD exposures, we identified low delta-band power as a candidate neural biomarker of OC symptom intensity during symptom provocations in one participant (left VC/VS: R=-0.59, p=0.01; right VC/VS: R=-0.56, p=0.04). Electrophysiological analysis of acute response to stimulation revealed an increase in 30 Hz power specific to lateral OFC that was associated with increased talkativeness and approach behaviors. Over time, the focal 30 Hz signal stabilized, coinciding with an inflection point in clinical scores including a reduction in OCD severity. These signals have potential utility for classification of symptom intensity and increased mood and energy to enable adaptive DBS systems for OCD. Our data suggest that the neural biomarkers of these two competing states might be different, which will allow for simultaneous optimization of both states.

Poster #28

H. Cho, M. Buchheit, C. A. Gkogkidis, C. Stolle, S. C. Cramer, J. Ojemann, J. Herron

Design and demonstration of an investigational research platform for phase-triggered cortical stimulation

By considering neural oscillations and their dynamic nature, adaptive stimulation may be more effective in modulating brain connectivity. This greater control over connectivity may indicate that adaptive stimulation approaches have the potential to better investigate the mechanisms underlying neurological conditions and inform stimulation design, resulting in improved therapeutic outcomes compared to open-loop techniques. With growing advances in sensing and stimulation technologies, there has also been a rising interest in the investigation of closed-loop methods. While such improvements have facilitated the progress of novel stimulation therapies, current clinical systems enable limited exploration of the adaptive stimulation design space. Here, we utilize the OMNI-BIC, an open-source software tool to enable customizable stimulation design with the CorTec Brain Interchange (CorTec GmbH, Freiburg, Germany), an upcoming investigational research device, to perform phase-locked stimulation. We implemented a processing pipeline within the OMNI-BIC to identify signals within a certain frequency band and send stimulation during a specific phase of the target signal. The pipeline incorporated elements, including real-time stimulation artifact mitigation to minimize contamination of neural signals, and a phase-locked loop design to dynamically trigger for more robust stimulation delivery. These signal processing components were included to minimize mistimed and over stimulation that could cause undesired effects. This phase-locked algorithm was then demonstrated within two sheep models to determine the feasibility of performing phase-locked stimulation with the OMNI-BIC. Implanted electrocorticography arrays were employed to record neural activity and deliver stimulation pulses. Collected resting state data was used to inform parameters, such as filter coefficients, target frequency, and trigger threshold. We characterized phase-locked stimulation performance across varying stimulation amplitudes for each animal model. Our findings show the OMNI-BIC and Brain Interchange ecosystem is capable of delivering phase-locked stimulation with limited instances of over stimulation, demonstrating the translatability of the OMNI-BIC from benchtop to clinical conditions.

These results also illustrate that the customizability of the OMNI-BIC enables greater design control to develop and assess other phase-triggered stimulation or adaptive neuromodulation paradigms.

Poster #27

Erynn Sorensen, Roberto de Freitas, Luigi Borda, Nikhil Verma, Peter Gerszten, Douglas Weber, Elvira Pirondini, George F. Wittenberg, John W. Krakauer, Marco Capogrosso

Exploring the interaction of residual corticospinal connections and spinal circuitry during stimulation of the cervical spinal cord post stroke

We have recently demonstrated that epidural spinal cord stimulation (SCS) of the cervical spinal cord enables recovery of upper limb motor control in post-stroke hemiparesis. However, the corticospinal mechanisms involved in SCS-based motor improvement are poorly defined for stroke. Here, we have implanted two chronic stroke subjects with epidural leads along the dorsal roots of the cervical spinal cord to investigate mechanisms of interaction between SCS and residual corticospinal connections. Interestingly, there have been no reports of improved muscle-evoked potentials (MEPs) in SCS study participants; suggesting that none of the clinical improvements detected in the absence of SCS are mediated by strengthening of the corticospinal tract's (CST) monosynaptic connections to the motoneuron. Rather, we posit that the CST primarily engages spinal circuitry pre-synaptically which, in turn, conditions the motor response to SCS. As such, we initiated a paired-pulse protocol wherein we assessed the effect of cortical conditioning (via single pulses of transcranial magnetic stimulation (TMS) over the impaired half of the motor cortex) on single pulses of SCS-evoked spinal reflex potentials (SRPs) of the arm and hand. Inter-pulse-intervals (IPIs) ranged from 5 to 400ms in length. We found that TMS conditions SRPs at both short and long latencies, even in an MEP negative subject. Surprisingly, we further found evidence that the greatest potentiation occurs with an IPI of approximately 50-150ms, which is incompatible with latencies corresponding to monosynaptic coincidence on the motoneuron. Rather, this timing suggests activation of interneurons via residual CST projections that serve to excite or disinhibit the sensory-motor reflex arc. Furthermore, we investigated the effect of residual descending motor commands to SCS-mediated spinal dynamics in passive and active motion. Subjects were configured in a robotic dynamometer and guided through 60 degrees of elbow flexion and extension while we delivered test pulses of SCS to determine innate SRPs. We then delivered 40Hz continuous stimulation and discovered that residual supraspinal connections could overwrite spinal dynamics during active motion, indeed completely reversing them during functional SCS; suggesting that SCS enables the post-stroke brain to actively modulate spinal reflexes as a form of motor control. Overall, this evidence suggests that residual CST projections interact pre-synaptically with spinal circuits and SCS to modulate muscle responses and illuminates the potential for complex mechanisms of interaction between residual, but seemingly nonfunctional, CST fibers and SCS.

Poster #49

Alex Baldwin, Victor Pikov, Raja Hitti, Ellis Meng

An open-source platform for clinical autonomic neuromodulation therapies

The field of bioelectronic medicine is rapidly advancing, and new therapies are being proposed which require careful closed-loop modulation of neural activity in peripheral nerves including the vagus nerve, sacral nerve, pudendal nerve, enteric nervous system, and others. However, translating a neuromodulation therapy to human clinical use is difficult due to the high cost and long timelines involved in developing human ready implantable devices. Today, most clinical research groups use established commercial devices for large animal and human trials; these devices are generally designed for specific indications and may not meet the needs of the new therapy, and working with larger

companies can be challenging due to conflicts of interest which may arise that interfere with successful partnerships. In addition, existing devices often do not have the ability to record biomarkers from multiple sites for active stimulation, limiting therapeutic potential. Access to customizable neuromodulation technology is therefore a major barrier to new discoveries as well as development of new therapies. The CARSS Center is a public-private partnership between the University of Southern California, Medipace Inc., and Med-Ally LLC, which is changing this by building an open-source system for closed-loop autonomic neuromodulation aimed at human clinical studies. The CARSS system is composed of an implantable pulse generator (IPG) with Bluetooth capabilities and the ability to run machine learning algorithms natively, and a variety of leads for stimulation and sensing. The first generation leads include a vagus nerve cuff and sacral nerve linear array with both stimulation and neural sensing capabilities, and electrocardiography, electromyography, and motion sensing leads for physiological monitoring. Additional leads under development include sensing leads for neurotransmitters including acetylcholine and catecholamines and physical sensing leads for strain and end-organ temperature. CARSS is currently seeking partners who are interested in using the system to conduct large animal studies for specific indications that will lead to a first in human use.

Poster #51

Kevin Wilkins, Emilia Lambert, Jillian Melbourne, Matt Petrucci, Aryaman Gala, Pranav Akella, Laura Parisi, Sudeep Aditham, Gerrit Orthlieb, Chuyi Cui, Jeff Herron, Helen Bronte-Stewart
Beta burst driven closed loop deep brain stimulation for freezing of gait in Parkinson's disease

Background: Freezing of gait (FOG) is a debilitating symptom of Parkinson's disease (PD) that is often refractory to medication. Pathological prolonged beta bursts within the subthalamic nucleus (STN) are associated with both worse impairment and freezing behavior in PD. Deep brain stimulation (DBS) improves FOG and shortens burst durations. Therefore, the goal of the current study was to investigate the feasibility, safety, and tolerability of beta burst driven closed-loop deep brain stimulation for FOG in PD.

Methods: Seven individuals with Parkinson's disease were implanted with the investigational Summit RC+S DBS system with leads placed bilaterally in the STN. A PC-in-the-loop architecture was used to adjust stimulation amplitude in real-time based on the observed beta burst durations in the STN. Patients performed either a harnessed stepping-in-place (SIP) task or a free walking turning and barrier course (TBC) OFF stimulation, on closed-loop, open-loop, or a randomized intermittent open-loop DBS. Overall motor impairment was measured as well as quantitative gait metrics across each condition.

Results: Beta burst driven closed-loop DBS was successfully implemented and deemed safe and tolerable in all eight participants. Both overall motor impairment and gait metrics were improved from OFF to closed-loop DBS, which showed similar efficacy as open-loop.

Conclusion: Beta burst driven closed-loop DBS was feasible, safe, and tolerable in individuals with PD.

Poster #22

Jude Savarraj, Samden Lhatoo, GQ Zhang, Yosefa Modiano, Jamie Van Gompel, Behnaam Aazhang, Sandipan Pati, Gregory Worrell, Nitin Tandon
Network neuromodulation for medial temporal lobe epilepsy

Refractory medial temporal lobe epilepsy (MTLE) impacts close to a million individuals across the United States. In a substantial proportion (~40%) of patients with refractory focal epilepsy, seizures originate in

the medial temporal lobe where they adversely impact the neural substrates for episodic memory. Traditional interventions, such as resective or ablative surgery, carry the risk of memory impairment due to their impact on critical areas including the hippocampus and para-hippocampal gyrus. Our study introduces an innovative approach, viewing MTLE through the lens of a network disorder characterized by hyperexcitability, and suggests that this can be addressed by applying sub-threshold, low-frequency stimulation to the limbic network. Although this technique has been successful in decreasing hyperexcitability in animal models, its systematic application in human epilepsy has not been adequately investigated.

The research involves a meticulously designed two-stage protocol. Initially, we identify patients with drug-resistant epilepsy originating in the dominant hippocampus who retain memory function and are undergoing standard intracranial monitoring using stereo-electroencephalography (SEEG). This stage involves detailed recordings from mesial temporal structures, including the amygdala, hippocampus, entorhinal cortex, and extending to the temporal neocortex, insula, and orbito-frontal cortex. These recordings are crucial to accurately localize the seizure onset zone. Additionally, we examine the anterior nucleus of the thalamus (ANT) for its contributions to the seizure network. With this data, we can customize stimulation parameters to the individual's neural activity patterns, identifying the most effective settings within safe operational boundaries. The next phase involves the application of the Medtronic PerceptTM PC System equipped with BrainSenseTM Technology, which will target the hippocampal fornix, piriform cortex, entorhinal cortex, and ANT for persistent low-frequency stimulation. The objective is to disrupt epileptic hypersynchrony, thereby reducing seizure frequency and examining the potential benefits on memory performance.

Our interdisciplinary team, comprising specialists from UTHealth, Mayo Clinic, and Rice University, is dedicated to establishing a reproducible model of the MTLE network and exploring the stimulation parameter space to find the most beneficial neuromodulation approach. Additionally, we will assess the safety and practicality of the Percept PC system in the autonomous detection of seizures and the generation of an electronic seizure diary. We anticipate that our network-focused neuromodulation strategy will lead to a significant decrease in seizure frequency and severity, reduce inter-ictal spikes, and yield cognitive enhancements, specifically in memory functions. This novel treatment could significantly alter the current landscape of MTLE management, offering new therapeutic avenues for those constrained by the existing limits of epilepsy treatment.

Poster #60

Huijing Xu, Yingyi Gao, Tuo Zhou, Ellis Meng,Dong Song

Development of a design library of polymer-based microelectrode arrays for rodent multi-region hippocampal recordings

Obtaining long-term, stable recordings from anatomically and functionally connected brain regions of behaving animals is the foundation for studying brain functions. As one essential tool, conformal penetrating polymer microelectrode arrays (pMEAs) enabled direct access to individual neurons in multiple brain regions. Recently, advances in pMEAs demonstrated year-long recordings from neurons in rodent brains. However, access to pMEAs is greatly limited to selected groups with advanced nano-fabrication capabilities. To broaden the accessibility and promote the development of pMEAs, we are developing a library of pMEA designs covering various cortical and sub-cortical regions of the brains of mice, rats, and nonhuman primates (NHPs). We start with the hippocampus, a brain region that is of great interest in neuroscience and neural engineering and has a complex anatomical structure with distinct subregions. Two 3D hippocampal multi-region pMEAs are designed for rats. Design A consists of

two four-shank MEAs with 64 channels in each MEA. One MEA targets the CA1 and the dentate gyrus (DG) subregions, while the second MEA, which is 1500 μm lateral to the first one, targets the CA1 and the CA3 subregions. Electrodes on each shank are divided into two recording groups with electrode layouts conforming to the curvature of hippocampal cell body layers. Design B consists of four two-shank MEAs with 32 channels in each MEA. Two MEAs are assembled back-to-back to enable double-side recordings. Like design A, the MEAs conform to the DG, CA3, and CA1 subregions. For mice, design A and B are reduced to four-shank 32-channel MEAs conforming to the cell body layers on a coronal hippocampal plane. These pMEA designs will be fabricated and delivered to expert users for reviews and feedback. Optimization of the pMEAs will be performed iteratively. Implantation methods for pMEAs with different lengths (5 to 40 mm), e.g., dip-coating and insertion shuttles, will also be developed and evaluated.

Poster #36

Brenda J. Yu, Afik Faerman, Yun Xiang, Mahaveer P. Purohit, Kanchan Sinha Roy, Matine M. Azadian, Nolan Williams, Raag D. Airan

Development and clinical translation of ultrasonic drug uncaging for precision neuropharmacology

Ultrasonic drug leverages recent advances in therapeutic ultrasound systems that can target sonication to millimeter-sized regions of interest throughout the body and are in active clinical use for varied applications worldwide. It combines that spatial precision with the power of neuropharmacology to promise millimetrically-precise noninvasive treatment of the nervous system for neurologic and psychiatric applications. In this program, we have worked to translate ultrasonic drug uncaging from our previous preclinical, proof-of-concept data into a form suitable for application in human patients. We pursue two different first-in-human trials of ultrasonic drug uncaging: targeted anesthesia of putative epileptogenic targets to determine their contribution to treatment resistant epilepsy; and targeted ketamine delivery to the anterior cingulate cortex in patients with chronic pain.

For the ketamine program, we have developed an entirely new system for ultrasonic drug uncaging in which acoustomechanically-activatable liposomes (AALs) loaded with the drug of interest release drug with ultrasound application, with their ultrasound responsiveness determined by the acoustic impedance and osmolarity of the liposome internal medium. *In vitro*, we have demonstrated we may indeed tune the ultrasound-responsiveness of AALs by altering the acoustic impedance of the particle internal medium. We have further shown that AALs permit ultrasonic drug uncaging to the brain, with a resolution of <5mm using 250 kHz ultrasound, and that the clearance profile of the uncaged drug matches the expected profile for ketamine, with minimal residual in the brain after 15-20 min post uncaging. We have further confirmed the safety of AAL-mediated ultrasonic drug uncaging, seeing no evidence of direct parenchymal injury, neuronal degeneration, inflammation, or gliosis relative to sham controls.

For a functional evaluation we used rat electrocorticography (ECoG) as an analogue of the scalp EEG we intend for our first-in-human trials. Using rat ECoG, we confirmed that ketamine AAL ultrasonic drug uncaging recapitulated known effects of ketamine delivery drawn from rat and human electrophysiologic analysis following ketamine administration. Furthermore, by shifting the sonication focus between medial prefrontal cortex (mPFC) and retrosplenial cortex (RsC), sites of interest regarding the affective (mPFC) and dissociative (RsC) actions of ketamine, we note different patterns of ECoG resolved activity with mPFC sonication yielding a more pronounced gamma band induction and RsC sonication yielding a more pronounced theta band induction, confirming patterns from human intracranial recordings, and confirming the functional site specificity of ultrasonic ketamine uncaging.

Curiously, ultrasonic ketamine uncaging yielded more powerful and sustained effects compared to dose-matched infusions of free ketamine.

As AALs are composed of relatively common pharmaceutical excipients, they have a low barrier to clinical translation and we are rapidly moving towards a first-in-human trial in which we will target ketamine delivery to the dorsal anterior cingulate (dACC) as doing so has been hypothesized to be effective in treating the affective component of chronic pain. Patients will undergo screening, baseline, treatment, and two post treatment visits during which clinical, pain, and blood lab assessments will be taken in addition to MRI/fMRI/MRS imaging. On the procedure day, with simultaneous EEG monitoring, patients will receive ultrasound alone followed by AAL infusion and then ultrasound application to the dACC. Blood samples will be taken for pharmacokinetic analysis and behavioral assessments will be taken to assess for efficacy of dACC ketamine uncaging to affect measures of pain, dissociation, or sedation. A dose-escalation paradigm will be used to assess for a maximum tolerated dose of ultrasonic ketamine uncaging in correlation to these efficacy measures, to guide subsequent Phase II studies.

Poster #37

Jeff Elias, Changchia Liu, Mark Quigg, Patrick Finan, Shayan Moosa
A clinical trial to investigate neuromodulation of the insula for chronic neuropathic pain

Background: The insula has been identified as a key component of pain processing in laboratory and imaging studies in both animals and humans. We have preliminary evidence that neuromodulation of the anterior insula increases the heat pain threshold in humans.

Methods: A two-staged clinical trial was designed to comprehensively investigate the insula as a target for neuromodulation to manage chronic neuropathic pain. Twelve subjects with severe, refractory, chronic neuropathic pain will be enrolled. Phase 1 involves stereotactic implantation of multi-contact depth electrodes longitudinally into the bilateral insula. Brain mapping will be performed in the hospital with continuous intracranial EEG and heart rate monitoring. Clinical pain ratings, quantitative sensory testing, and laser evoked potentials will be used to measure nociceptive responses to stimulation of each of the insular gyri. Subjects who respond to insular stimulation in at least one gyrus during the mapping phase will advanced to phase 2 for DBS implantation and a randomized controlled trial.

Results: Four subjects have been implanted with bilateral intracerebral depth electrodes. There have been no safety events with surgery. After discharges have been observed during brain mapping with higher currents. All subjects were deemed ‘responders’ during the trial phase, based on 50% decreased in clinical pain, greater than 2-points reduction in experimental pain on 0-10 scale, and/or greater than 2°C heat pain threshold increases following insula stimulation.

Conclusions: Stimulation of the insula is well tolerated in the hospital settings. Attenuated nociceptive responses to electrical stimulation of the anterior insula have been observed in the first four subjects with severe chronic neuropathic pain. The effects of chronic insular neuromodulation using DBS are currently under observation.

Poster #66

L. Rieth, I. Sondh, K.-H. Dyballa, M. Leber, J. Crew, K. HÃ¼bner, A. Heiller, W. Nogueira, L. Johnson, G. Ghose, W. M. Thomas, R. Gurgel, D. Warren, S. Zuniga, D. Chieffe, A. Loveland, L. Larson, R. Franklin, A. Samii, A. Oxenham, S. Strahl, C. Batsoulis, D. Sieber, F. Solzbacher, M. Adams, T. Lenarz, H. Lim
Pre-clinical testing of a penetrating auditory nerve interface

Cochlear implants (CIs) are the most successful sensory neural prosthetic to date with >1 million implanted, which have provided or restored meaningful hearing for patients. Considerable resources have been invested to improve CI performance in the last 25 years, but resulted in modest improvement for real-world tasks such as understanding speech in noise. Spreading of the stimulation current due to the low conductivity of bone between the electrode and nerve, and the conductivity of perilymph around the cochlear electrode are putative factors limiting the number independent channels. We have developed a penetrating electrode, the auditory nerve Utah slanted electrode array (AN-USEA) fabricated by Blackrock Neurotech. The array is surgically placed in the auditory nerve, and driven by a MED-EL SYNCHRONY 2 cochlear stimulator. Our study will evaluate if more selective stimulation can be achieved through an intraneural interface and safely generate more natural hearing in human subjects. We report the device architecture used for Auditory Nerve Implant (ANI) devices, and bench tests such as impedance measurements and stimulation characterization from fully functional devices. Pilot studies with wired ANIs to develop a chronic cat model were also performed, and an optimized surgical procedure was developed to maintain the health of the animals and stimulation performance of the ANIs. Animal health was maintained for longer than the 6-month endpoints to be used in preclinical studies. The electrode impedances increased with implantation but remained < 70 kΩ in at least one animal, which suggests current can be delivered effectively. Nerve engagement was evaluated by evoked auditory brainstem recordings (eABRs) collected longitudinally. At least one animal maintained very convincing eABRs for longer than the 6-month pre-clinical endpoint supporting the viability of the cat model, surgical approach, and electrode technology. These studies have also evaluated the activation thresholds, forward masking paradigms to help confirm auditory nerve fibers are recruited, and channel independence through masking with separate electrodes and achieved highly promising results. The surgical approach for NHP studies has continued to be developed, focusing on implantation, insertion, and stabilization methods, which are more technically demanding than previous human cadaver studies. The impedances of implanted electrodes and eABR measurements were used to evaluate the electrode-tissue interface and nerve engagement, respectively. Data from large animal models will be used to gain approvals for first-in-human implants of the ANI devices.

Poster #38

Joanna Lin, Jereen Kwong, Ryan Leriche, Thomas A. Wozny, Ana Shaughnessy, Ashlyn Schmitgen, Prasad Shirvalkar

Quantifying pain location and intensity with multimodal pain body diagrams

Pain is particularly difficult to capture and communicate due to its subjective, multidimensional nature spanning somatosensory, affective, and cognitive processes. Standard pain rating scales such as the numeric rating scale (NRS), visual analog scale (VAS), or McGill pain questionnaire (MPQ) are commonly used to quantify pain. However, these scales face response anchoring bias and often fail to capture complex pain experiences dispersed across body regions. In contrast, pain body diagrams (PBDs) visualize differential areas of pain. Previous versions of this tool were largely qualitative, but here we present a novel method that uses a pressure-hue transformation to visualize and quantify granular pain intensity and location data for 5 patients with chronic pain. Patients were instructed to apply different drawing pressures with a digital stylus on the PBD to output hues ranging from green to blue to red to represent mild to moderate to most painful regions, respectively. Three metrics from PBDs were extracted: (1) PBD mean intensity, which equals the sum of each pixel's hue value divided by the number of colored pixels, (2) PBD coverage, which equals the number of colored pixels divided by the total number of pixels on the body, and (3) PBD sum intensity, which equals the sum of all pixels' hue values. Using correlation analyses (n=609 PBDs), these PBD metrics were shown to be highly concordant with standardized pain metrics, including NRS, VAS, and MPQ. The PBD sum, coverage, and mean were

significantly correlated with VAS and NRS scores in four out of five participants (Spearman's correlation rs = 0.33-0.72, $p < 0.004$) and to MPQ scores in three out of five participants (Spearman's correlation rs = 0.38-0.53, $p < 0.004$). Additionally, information theory analyses revealed PBD metrics contain greater entropy compared to the NRS, demonstrating less response anchoring within the PBD method (Tukey's t-test for individual comparisons $p < 0.05$). Furthermore, four out of five participants reported that the method was easy to use and accurately reflected their pain. Thus, PBDs implementing a pressure-hue transformation provide combined spatial and quantitative information that can longitudinally measure and track pain to comprehensively characterize a patient's pain experience.

Poster #23

Paul Botros, Jihwan Lee, Keundong Lee, Tara Porter, Dongwoo Kim, Allen Munk, Hao Le, Gal Mishne, Louis Liu, Drew Hall, Xinyu Zhang, David Roth, Kiefer Forseth, Hoi Sang U, Angelique Paulk, Eric Halgren, Ahmed Raslan, Sydney Cash, Sharona Ben-Haim, Shadi Dayeh

Thin, high-density depth and surface microelectrodes with wireless recording and stimulation for diagnosis and treatment of epilepsy

Over 1% of the US population has epilepsy, a neurological disorder marked by recurrent seizures, and over 30% of these patients suffer from drug-resistant epilepsy (DRE), severely interfering with quality of life despite treatment advances. Resective surgery aims to treat DRE patients by removing epileptogenic brain tissue while sparing functional tissue, but today's process for delineation of epileptogenic vs functional tissue relies on clinical electrodes with poor spatial resolution (5-10mm contact pitch), potentially limiting the accuracy of resective surgery. Furthermore, today's solutions require numerous wires to exit the head, bulky amplifiers, and connecting systems, increasing infection risk and patient discomfort. Here, we aim to advance acute and semi-chronic epilepsy monitoring using novel, high-resolution electrocorticography (ECoG) grids (4096/256 record/stimulate channels, respectively) and stereoelectroencephalography (sEEG) depth electrodes (128/16 micro/macro). These novel ECoG grids and sEEG probes will be integrated into a fully or partially implanted system with wireless power and data transmission. In-house visualizations utilize real-time data processing to display clinically relevant neural data for clinicians in both intraoperative and semichronic settings. Achieving fully wireless high-channel-count neural electrode systems, high spatial resolution, multi-thousand channels, and integrated real-time visualization systems may have a substantial impact on patient outcomes and clinical procedures.

To this end, first, we scaled our ECoG grids to 4096/256 record/stim channels and our sEEG electrodes to 128/16 micro/macro contacts, demonstrating high yield and good functionality in both benchtop and *in vivo* settings. Second, we implemented a head-worn unit, responsible for wireless data recording and stimulation, and demonstrated *in vivo* end-to-end wireless data acquisition with high throughput, low latency, and submillisecond time synchronization. In parallel, we implemented parts of the planned subcutaneous system, validating wireless power and data transmission through the scalp. Finally, we developed software to handle high-channel-count recording, leaning on clinician input. Ongoing efforts include full system integration with testing in benchtop and acute and chronic pig models, enclosure and form factor development, and continued regulatory discussions with the FDA.

Poster #61

J. Olson, S. Wahid, Z. Irwin, D. Kuhman, C. Gonzalez, M. Boolos, S. Black, B. Guthrie, T. Wichmann, H. Walker

Subthalamic nucleus single-unit activity in humans with Parkinson disease encodes both the rate of change and magnitude of force during a sustained grip-force task

The subthalamic nucleus (STN) is a primary target for Parkinson disease (PD) neuromodulation therapies, yet its role in sensorimotor neural circuits remains unclear. We investigated the neuronal activity in the dorsolateral STN during a visually cued isometric grip-force task in the contralateral hand in humans with PD who were undergoing deep brain stimulation surgery. We found significant force-related changes in STN unit activity, especially immediately after force onset and offset, but minimal changes during movement preparation. The most substantive changes in single-unit activity occurred with changes in grip force (15 of 21 units, 71%) rather than during sustained grip (12 of 21, 57%). During sustained force, the neuronal spike frequency varied linearly with force magnitude in seven units (33%), and the spike frequency during the applied force differed from baseline in nine (43%) units, all clustered in the dorsolateral STN. During a phase of the task when force changes, six units increased their spiking, while four units decreased their activity. Units with increases in activity discharged at a median of 56.5 (IQR: 25 to 156) ms after mechanical squeeze onset, whereas units with decreases preceded squeeze onset by 69.5 (23 to 79) ms. When force was released, five units increased their activity, while eight units decreased their activity, at latencies of 153.0 (84 to 260) ms and 58.0 (38 to 76) ms after release onset, respectively. Our findings suggest that neurons in the dorsolateral STN encode dynamic changes in force to a greater extent than sustained force magnitude and may play greater roles in sensory feedback and movement refinement rather than movement preparation.

Poster #39

David Houghton, Bashar Badran, Navid Khodaparast, Kathryn Cunningham, Denise Wilkes

Transcutaneous auricular neurostimulation for chronic pain and opioid withdrawal

Due to surging rates of opioid misuse and overdose deaths, recent clinical guidelines recommend reducing doses or using alternative treatments for long-term prescription opioid-maintained patients. However, for the ~8.5 million Americans who depend on regular opioids to control chronic pain, cessation can be highly aversive and challenging to accomplish. Even when tapering slowly, many patients have concerns about uncontrolled pain, and forced tapering has been linked to treatment drop-out, mental health crises, and suicide. We therefore need methods to effectively control pain while mitigating opioid withdrawal. Transcutaneous auricular neurostimulation (tAN), a novel neuromodulation approach that noninvasively stimulates the vagus and trigeminal nerves in the ear has shown success in rendering analgesia during opioid withdrawal. Unfortunately, the mechanism for the anti-pain effects of tAN are unclear. In order to optimize tAN therapy and prepare for real-world implementation, the mechanism of action must be fully characterized. Through a series of parallel and complementary studies funded by the Heal Initiative, our team is currently conducting a parallel series of investigations that include a mechanistic clinical trial of the neurophysiologic effects of tAN during opioid withdrawal. Using a randomized, double-blind, sham-controlled design, we are enrolling 40 chronic pain patients on long-term opioid prescriptions. During a four-day inpatient stay, patients undergo moderate tapering of their opioid medication while receiving either active or sham tAN. Subjects undergo baseline and post-treatment assessment as well as functional neuroimaging. Early results reveal some patients reporting remarkable results, such as decreased chronic pain severity, reduced depression and anxiety symptoms, improved physical functioning, and abatement of opioid withdrawal. We will discuss plans for later analyses, particularly with functional neuroimaging, as well as implications of results in concert with concurrent research.

Poster #76

Sarah A. Brinkerhoff, Joseph W. Olson, Mohammad Awad, Cameron Gordon, Christopher L. Gonzalez, Arie Nakhmani, Nicole Bentley, Marshall T. Holland, Bart L. Guthrie, Harrison C. Walker
Paired deep brain stimuli elicit short-term facilitation in globus pallidus interna and subthalamic nucleus

High frequency deep brain stimulation (DBS) is a highly effective neurosurgical treatment for Parkinson's disease. Despite its efficacy, knowledge about how DBS interacts with local brain circuitry is limited. Paired DBS pulses elicit local event-related electrical potentials near the stimulation site in the subthalamic nucleus (STN), the globus pallidus interna (GPi), and the ventral intermediate thalamus (VIM). These event-related potentials occur across all targets at <0.5 milliseconds latency after stimulus onset and presumably represent direct depolarization of proximal neural elements by the stimulus pulse. Subsequent oscillatory event-related potentials, termed evoked resonant neural activity (ERNA), occur at longer latencies (~4 ms) in STN and GPi but not in the VIM thalamus. Greater knowledge on the fast dynamics of ERNA could shed light on mechanism of action, disease pathophysiology, and novel biomarkers to guide DBS therapy. Here, we contrast ERNA features in the STN versus GPi, the canonical functional targets for Parkinson's disease. We hypothesized that ERNA amplitude, temporal dynamics, and number of peaks differ in STN (n=14) versus GPi (n=12). We delivered pairs of DBS pulses across a range of interstimulus intervals and amplitudes during surgery and recorded local evoked potentials from non-stimulating bipolar configurations on the implanted lead. Following artifact removal, we contrasted ERNA amplitude, frequency, onset latency, offset latency, wave period, and number of peaks by interstimulus interval and brain target. STN and GPi DBS both elicit ERNA, but ERNA amplitude was considerably larger in STN than in GPi (p=0.001). Otherwise, ERNA displayed similar onset latencies, offset latencies, peak-to-peak frequencies, wave periods, and number of peaks across targets. In conclusion, pairs of DBS pulses elicit larger amplitude ERNA responses in STN than in GPi. The presence of ERNA in these targets (but not the VIM) suggests a role in the pathophysiology of a tremulous motor features of Parkinson's disease and dystonia. These and other evoked local field potential responses could eventually guide open-loop DBS programming or serve as control signals for adaptive therapy.

Poster #24

Amir Hossein Ayyoubi, Behrang Fazli Besheli, Michael Quach, Jay Gavvala, Alica Goldman, Chandra Prakash Swamy, Eleonora Bartoli, Daniel Curry, Sameer A.Sheth, David J. Francis, Nuri F.Ince
The early feasibility of recording intracranial EEG and epileptic spikes with the brain interchange system

The use of implanted pulse generators for neuromodulation has proven to be a promising method for treating neurological disorders. For instance, responsive neural stimulation (RNS) for the control of seizures in epilepsy and deep brain stimulation (DBS) in movement disorders. However, the implantable systems face various challenges, including recording only from a small number of brain sites, power management, and limited access to the assessed neural data in a continuous fashion. In this study, we investigated the feasibility of recording neural data from three human subjects with refractory focal epilepsy using a wireless, externally powered, portable bio-signal amplifier, the Brain Interchange (BIC) system (CorTec GMBH, Freiburg Germany).

We established a MATLAB/Simulink environment to acquire the neural data at 1kHz from the 32 channels of the BIC system and visualize the captured neural activity in real-time. Afterward, the established environment was subjected to validation in a real-world setting, where the intracranial EEG (iEEG) data was split into two streams and simultaneously recorded using a clinical amplifier and BIC system within the epilepsy monitoring unit (EMU). Subsequently, the raw signal quality and background noise characteristic of each stream were quantified and compared. Although the clinical amplifiers

utilizing a cable interface exhibited a significantly better noise floor (<25 dB), the BIC system demonstrated the capability to collect data of comparable quality wirelessly and continuously. The primary distinctions in noise floor between the two systems were predominantly observed above 100 Hz.

Furthermore, the recorded data for each stream (BIC vs. Natus Quantum (Natus Medical Incorporated, Wisconsin, USA) and BIC vs. Nihon Kohden (Nihon Kohden Corporation, Tokyo, Japan)) underwent a spike detection analysis, ensuring similar/aligned time segments. The results showed a concordance of over 90% between the two systems. As a result of the BIC system's wireless data transfer capability, we may face losing the packets of data during the transfer process (Packet loss). We estimated the packet loss level of the recording interval for each subject. Moreover, to further investigate the effect of ambient noise on the packet loss, we conducted a series of tests in the lab and the electromagnetically shielded chamber, which revealed that there is a significant difference between the two environments (paired t-test, $p = 0.0090$) with an average of 1.47% packet loss in the lab and 0.74% in the shielded chamber.

Acknowledgements: This study was supported by grants R01NS112497 and UH3NS117944 from the National Institutes of Health - National Institute of Neurological Disorders and Stroke.

Poster #25

Nikhil Verma, Erick Carranza, Erynn Sorensen, Luigi Borda, Marc P. Powell, Roberto M. De Freitas, Amy Boos, Peter Gerszten, George F. Wittenberg, Lee Fisher, Elvira Pirondini, Marco Capogrosso, And Douglas J. Weber

Closed-loop control strategies for improving the effects of spinal cord stimulation on motor recovery after stroke

Stroke damages the corticospinal tract, causing motor impairments that are often permanent. Recently, we demonstrated that tonic epidural stimulation of the cervical spinal cord improves upper limb motor function, specifically strength and dexterity in the arm and hand of people with chronic hemiparesis post-stroke. While tonic stimulation enhances the recruitment of targeted muscles, manual tasks of daily life involve coordinated and sequential activation of muscles throughout the limb. Previous studies of SCS in animals have shown that phase-specific patterns of SCS were required to enable compound movements comprising reaching, grasping, and retraction phases.

Four participants with chronic post-stroke hemiparesis were implanted with two 8-contact percutaneous SCS leads in the epidural space of the cervical spinal cord, ipsilateral to the paretic arm, for a duration of 30 days. SCS was delivered using a custom-built stimulation system that allowed for real-time control of the amplitude and frequency of stimulation applied to each contact. Participants performed planar and 3D reaching and grasping movements with their affected limb with and without SCS. Tonic and phasic stimulation was applied during the reaching tasks. During tonic SCS, the stimulation parameters were fixed and delivered continuously throughout the different phases of movement. During phasic SCS, stimulation was applied to different groups of electrodes during the extension and flexion phases. Furthermore, stimulation amplitude and frequency were modulated in real-time based on arm position relative to the target.

The participants were able to perform the task faster and with smoother trajectories with tonic SCS than without. Phasic SCS further improved reaching kinematics by increasing maximum hand speed, reducing movement duration, and smoothing hand trajectories. An up to 150% increase in muscle activations for phasic SCS was measured compared to the tonic and the no SCS conditions. These results show that

although tonic SCS was effective in promoting significant gains in motor function, phasic SCS produced even stronger effects.

Poster #40

Rawan Fakhreddine, Darcy Griffin, Nikhil Verma, Erynn Sorensen, Erick Carranza, Amy Boos, George Wittenberg, Peter C. Gerszten, Elvira Pirondini, Marco Capogrosso, Douglas J Weber
Spinal cord stimulation in stroke patients suppresses compensatory activity during movement

Despite stroke being a leading cause of serious long-term disability, post-stroke rehabilitation is often unable to promote recovery of arm and hand function. Patients with chronic hemiplegia often use compensatory strategies in the unaffected limb to augment function in the affected limb. These strategies can be in the form of mirror movements (unaffected hand displays a scaled version of affected hand activity) or general compensatory movements (reliance on trunk or abdomen) to better perform a task [1]. Recently, we demonstrated that cervical epidural spinal cord stimulation (SCS) targeting the dorsal root entry zone improves upper extremity function in people with stroke related hemiplegia. The effects of SCS are attributed to activation of primary afferent neurons that provide excitatory input to motor neurons. This may compensate for the loss of descending input after stroke [2] and reduce the need for compensatory strategies. To test this hypothesis, we examined EMG and reach-related kinematics in a patient with chronic stroke related hemiplegia during cervical epidural SCS.

We instructed the subject to perform a point-to-point reaching task in a 2-D plane with their affected limb. A session consisted of trials with (stim on) and without (stim off) SCS. Although the task required only movement of the affected limb, we recorded EMG and kinematics from both limbs. Our analysis focused on the biceps and triceps of both arms, revealing consistent patterns of muscle activation in the unaffected arm while reaching with the affected arm. We found signs of mirroring in the EMG and kinematics, by means of path length analysis and trajectory traces. Averages of EMG activity revealed that activation of the tricep in the affected limb activated with the tricep of the unaffected limb with no latency during the reach phase of the task.

During stim off trials, the participant often failed to reach the left and right targets. Motor performance of the affected limb improved with stim on, as seen by smoother kinematic traces, increased success rate, and shorter trial times. As motor behavior improved, the need for compensatory action decreased, revealed by reduction of average EMG activity in biceps and triceps of the unaffected side during stim on trials. EMG traces in individual trials also revealed lower levels of muscle activity in the unaffected limb with SCS, while in the affected limb EMG activity became more closely locked to reach onset with clearer activation peaks. Our analysis further reveals that SCS improves motor performance and reduces the need for compensatory behavior.

Poster #41

Luigi Borda, Erynn Sorensen, Erick Carranza, Roberto M. de Freitas, Nikhil Verma, Amy Boos, George F. Wittenberg, Peter Gerszten, Lee E. Fisher, Elvira Pirondini, Marco Capogrosso, Douglas Weber
Epidural spinal cord stimulation reduces agonist-antagonist co-contraction facilitating strength production

Stroke is the leading cause of long-term disability in the United States. Our group has recently demonstrated that cervical spinal cord stimulation (SCS) targeting the primary afferents lead to immediate improvements in muscle strength, kinematics and functional movements in people with post-stroke chronic hemiparesis. Although SCS is known to preferentially recruit large-diameter sensory afferents in the dorsal column and dorsal roots, the mechanisms underlying the immediate

improvements in motor function observed with tonic stimulation remain unclear. Here, we tested the hypothesis that SCS reduces co-activation of antagonistic muscles by reciprocal inhibition. We hypothesized that the gain of the monosynaptic reflex measured in the antagonist muscles would be suppressed with SCS targeting the agonist muscle. Moreover, we hypothesized that these effects may exist even when SCS was delivered during passive movements (e.g., does not produce voluntary movements) indicating the direct, inhibitory effects of SCS on reflex gains in antagonistic muscles.

Two 8-contact SCS leads were implanted percutaneously in the dorsal epidural space of the cervical spinal cord ipsilateral to the paretic arm of an individual with a unilateral subcortical stroke. Myoelectric activity was recorded from an antagonistic muscle pair, specifically biceps brachii (BB) and triceps brachii (TB). We then measured the amplitude of reflex responses evoked by SCS under active and passive conditions. In the active condition, the subject performed an elbow extension movement using a robotic exoskeleton. Tonic SCS was delivered at 40, 60 or 80 Hz on an electrode targeting facilitation of TB. A second electrode was used to elicit posterior root muscle reflexes (PRM) in the BB by delivering SCS concurrently at 2 Hz. In the passive condition, the experiment was repeated with the subject resting passively with the elbow extended at 180 degrees. In both conditions, the amplitude of the 2 Hz stimulation was sufficient to elicit the muscle response (PRM-reflex).

Our results show suppression of the PRM reflex elicited in BB when continuous SCS was delivered to facilitate TB in both the active and passive conditions. The inhibitory effects were evident across all SCS frequencies that were tested. Importantly, the inhibitory effects diminished when SCS was delivered through a contact that was not targeting the agonist muscle.

Our results support the hypothesis that SCS can actively inhibit monosynaptic reflex gains in antagonistic muscles. The direct suppression of reflex responses in opposing muscles could reduce agonist-antagonist co-contraction and increase the net torque produced at the joint

Poster #65

Kriti Kacker, James Bennett, Peter E Yoo, Abbey Sawyer, Nikole Chetty, Ashley N. Dalrymple, Devapratim Sarma, Dailyn Despradel, Adam Fry, Noam Harel, Shahram Majidi, Raul G. Nogueira, Jennifer L. Collinger, Nicholas L Opie, David Lacomis, Thomas J Oxley, David F Putrino, Douglas J Weber

Spectral features of endovascular ECoG signals recorded from a Stentrode in the human motor cortex

The Stentrode™ is a novel endovascular brain-computer interface (BCI) technology implanted to measure field potentials, similar to electrocorticography (ECoG), from the primary motor cortex to enable communication after severe paralysis. However, the features of these neural signals have not been fully characterized in humans. Participants with severe paralysis due to amyotrophic lateral sclerosis (ALS) and brainstem stroke have been implanted in pilot clinical trials in Australia (n=4) and the United States (n=4). The first in-human study was conducted in Australia, followed by an ongoing early feasibility study at 3 sites in the United States to evaluate the Stentrode's safety.

We aimed to identify robust spectral features for decoding the field potentials. Participants performed motor mapping experiments where they were instructed to attempt moving a specific body segment when prompted. The recorded field potentials were filtered into 3 frequency bands: beta (12-30 Hz), gamma (30-80 Hz), and high gamma (80-200 Hz). For each of the 3 band-limited signals, we calculated the change in root-mean-square voltage (Vrms) between rest and movement epochs, quantifying the percentage change of Vrms movement from rest (termed as modulation depth) for each trial. Principal component analysis (PCA) was used to merge signals that were correlated across channels. We also

assessed the signal stability of the Stentrode over a 99-day period, by measuring power of every channel for each frequency band.

We investigated the features of the Stentrode signals and identified the spectral characteristics that exhibit strong changes in amplitude between rest and attempted movement conditions. The high gamma frequency band exhibited the strongest modulation depth, showing ~100% increase in signal amplitude between rest and movement epochs. Analysis of somatotopic differences exhibited the strongest depth of modulation in the 'both ankles' and 'right hand' attempted movements. The signal power varied approximately ±0.5 dB in the high gamma frequency band over the 99-day period, which signifies high stability of the signal over time. The results of our preliminary analysis of the Stentrode signals indicate that these endovascular neural signals exhibit properties similar to those reported for ECoG-based measures of motor intent.

Poster #77

Nikole Chetty, James Bennett, Peter E Yoo, Abbey Sawyer, Kriti Kacker, Ashley N. Dalrymple, Devapratim Sarma, Dailyn Despradel, Adam Fry, Noam Y Harel, Shahram Majidi, Raul G. Nogueira, Jennifer L. Collinger, Nicholas L Opie, David Lacomis, Thomas J Oxley, David F Putrino, Douglas J Weber

Characterizing stability of human motor cortical activity recorded with a Stentrode

The Stentrode is a novel endovascular brain-computer interface (BCI) that is implanted within the superior sagittal sinus to record bilaterally from the primary motor cortices. The first-in-human trial in Australia demonstrated computer control and digital communication in four people with severe paralysis due to amyotrophic lateral sclerosis (ALS). An Early Feasibility clinical trial began in the United States (US) in July 2022 at three sites. In order for the Stentrode to be viable long-term, these endovascular electrocorticography (eECoG) signals need to remain stable over time to enable decoding of user intent. Multiple factors could contribute to signal instability and/or loss of BCI functionality in people with ALS, including neuronal degeneration and cortical atrophy, cognitive decline, inflammatory reactions, and device-related failures. Here, we explore the stability of eECoG signals recorded over 1 - 12 months in six participants.

To date, six participants with severe paralysis have been consented and implanted in the US, five diagnosed with ALS and one with brainstem stroke. Data acquisition and system training with the Stentrode began approximately 7 weeks after implantation. Each testing session begins with recording two-minutes of resting state activity, followed by training or utilization tasks. The offline (non-feedback) training tasks consisted of 5s (± 1 s) rest periods followed by a 5s period of movement attempt, in which 5 repetitions of attempted movement occurred. The movements attempted included: both ankles, right ankle, left ankle, right hand, left hand, and both hands.

Signal stability was assessed when participants were at rest or during motor attempts. The resting state signals were assessed through the root mean square (RMS) of the signal amplitude in the rest period, band power, and bandwidth. The attempted movements were evaluated using the movement (signal) and rest (noise) intervals for band power, percent change in RMS, and signal to noise ratio over the post-implant follow-up period to date. Band power was assessed in the standard frequency bands: alpha (8-13 Hz), beta (13-30 Hz), low gamma (30-60 Hz), and high gamma (60-200 Hz).

Results obtained thus far demonstrate that the eECoG signals recorded with the Stentrode are visually stable during the current follow up period (max 12 months to-date). Temporal linear regression will be

utilized to formally quantify any changes in slope during the follow-up period. The ongoing early feasibility study will continue to evaluate the signal stability beyond one year.

Poster #19

L. Osborn, M. Fifer, R. Franklin, D. Powell, P. Wanda, J. Monsen, B. Lindsey, P.E. Gaillardon, A. Zeinolabedin, M. Couriol, J. Zimmermann, T. Laabs, S. Keles, H. França, S. Montamat, D. Mathews, N. Crone, B. Wester, M. Wolmetz, F. Tenore

A Wireless, fully Implantable, bidirectional COrtical Neuroprosthetic System (W-ICONS)

The goal of this project is to develop a Wireless, fully Implantable, bidirectional Cortical Neuroprosthetic System (W-ICONS) for restoring sensorimotor function through an interface with upper limb areas of primary motor and sensory cortex. Technologies that enable direct communication to and from the brain have increasingly shown promise for restoring independence to people affected by high spinal cord injuries. Despite these advances, neural interface systems are still mostly confined to laboratory settings [1-3], requiring a team of researchers to handle cumbersome transcutaneous interfaces, extensive wiring, and bulky devices for recording and stimulating neural activity. Further, devices to date have typically been equipped with only neural recording capabilities []. The W-ICONS device would be the first wireless, bidirectional (incorporating neural recording and stimulation) cortical implant for individuals affected by tetraplegia, creating a truly portable device for use outside the lab environment.

Multiple/Other

Poster #78

Hannah Fuehrer, Khuleshwari Kurrey, Bo Ning, Lishan Lin, Maria Gabriela Mercado Guerra, Mali Jiang, Adnan Bibic, Peter C.M. van Zijl, Christopher A. Ross, Jun Hua and Wanli W. Smith
Neurovascular abnormalities in a G2019S-LRRK2 mouse model of Parkinson disease

Parkinson's disease (PD) is a common neurodegenerative disease characterized by motor impairments resulting from midbrain dopamine (DA) neuron loss. Mutations in LRRK2 cause genetic PD and contribute to sporadic PD. Here, we used G2019S-LRRK2-transgenic mouse model to investigate abnormalities in arteriolar cerebral blood volume (CBVa) and lymphatic vessels in various brain regions using the inflow-based vascular-space-occupancy (iVASO) and gadolinium-based MRI techniques. CBVa measured brain regions included in the substantia nigra (SN, the PD affected area), olfactory cortex and prefrontal cortex. Alterations in the blood volume of small arteries and arterioles (CBVa) were detected in the G2019S-LRRK2 mouse model of PD. Compared to non-transgenic mice, G2019S-LRRK2 mice at preclinical clinical stage showed increased CBVa and at clinical stage showed decreased CBVa in the SN in both male and female groups. On contrast, WT-LRRK2 mice showed no change in CBVa in the SN (male and female). Moreover, the G2019S-LRRK2 also induced abnormal dynamic signal changes of lymphatic vessels in the basal region (BR) compared with non-transgenic mice at both preclinical and clinical stages. These MRI changes in G2019S-LRRK2 mice was validated by pathological studies and was corresponding with PD pathology. Our results suggest the brain MRI changes of CBVa and lymphatic vessels may be useful as a marker for PD disease progression.

Poster #79

Mali Jiang, Ritika Miryala, Tianze Shi, Ronald Wang, Matthew Rodriguez, Karim Belkas, Rashi Sultania, Lauren Guttman, Kimberly Mae Bockley, Yushan Li, Anning Cui, Yuxuan Xue, Yuna Um, Ammy Yuan, Chloe Holland, Jing Jin, Juan C. Troncoso, Wenzhen Duan, Tamara Ratovitski, Wanli Smith, Christopher A. Ross
Inhibition of PKCα/ß1 kinase activity protects Huntington's disease human striatal neurons

Huntington's disease (HD) is a neurodegenerative disorder caused by a CAG repeat expansion in the huntingtin (HTT) gene. The repeat expansion leads to the pathogenic expansion of a polyglutamine tract in the huntingtin protein. HD is characterized by the loss of striatal medium spiny neurons and the presence of cellular inclusion bodies containing mutant HTT proteins. Currently, there is no disease-modifying treatment available for HD. In order to identify disease modifying treatment, we established a human neuronal model by immortalizing and differentiating HD patient iPSCs into highly homogeneous striatal precursor neurons (SPN), which recapitulated HD-like phenotypes of the parental iPSCs including expression of MAP2/DARPP32 (Akimov et al, Hum Mol Genet. 2021 Nov 30;30(24): 2469-2487). Further, we developed a 96-well plate screening platform using CellTiter-Glo luminescent cell viability assay in the SPNs and screened a kinase inhibitor library (ApexBio) containing 765 compounds in HD SPNs expressing 180 CAG repeats (180Q-SPNs). We identified approximately 20 compounds which exhibited protection to HD SPNs upon stress-induced neuronal toxicity.

Among the hits, we validated and prioritized a small molecule PKCα/ß1 inhibitor GO6976. We found that GO6976 dose-dependently rescued HD SPNs from stress induced toxicity. Furthermore, we examined PKCα/ß1 activity and protein levels in HD condition. The activity of PKCα/ß1 were significantly increased in HD ISPNs, mouse and human brains. Moreover, PKCα/ß1 interacted with both wild type and mutant HTT, and their overexpression was toxic to HD SPNs. These findings suggest that PKCα/ß1 may play

important roles in HD neurodegeneration and that inhibition of PKCα/ß1 activity may attenuate mutant HTT toxicity and provide novel therapeutic targets for developing neuroprotective HD treatments.

Poster #80

Jing Jin, Ainsley McCollum, Asvini Krishnaprakash, Nicolas Arbez, Chenwei Zhang, Stephen F. Martin, Christopher A. Ross

The role of Sigma 2 receptor/TMEM97 as a potential therapeutic target for neurodegenerative disorders

Huntington's disease (HD) is a devastating neurodegenerative disease caused by a single mutation in the huntingtin gene (*HTT*). The mutated *HTT* encodes a mutant protein (mHTT) with an expanded polyglutamine (poly Q), which affects the human striatum preferentially. HD pathogenesis includes mitochondrial dysfunction, axonal transportation deficits, abnormal transcription, metabolic dysfunction, etc., which are believed to be due to the gain of function of mHTT and loss of function of normal HTT. While the genetic cause has been known for decades, there is still no cure for HD. Gene therapies that target the cause of the disease lower abnormal mRNA, DNA, and protein of mHTT, but none of these strategies have succeeded because of poor brain penetration of agents and adverse events. Accordingly, therapeutic strategies using small molecules to reduce mHTT toxicity remain attractive for HD therapeutics. Our lab has set up an HD cell model that can be used to screen a variety of different compounds to rescue neuronal toxicity induced by mHTT. One set of compounds we tested is sigma-2 receptor (δ2R) binding ligands. δ2R binding ligands (modulators) have been used in cancer research for years. However, it was not until recently that its identity was confirmed as being the endoplasmic reticular protein known as transmembrane protein 97 (δ2R/TMEM97). Evidence indicated that δ2R/TMEM97 modulates cholesterol metabolism and lysosomal degradation by interacting with a cholesterol transporter protein, NPC-1. δ2R/TMEM97 may also be involved in ER stress response. Recently, δ2R/TMEM97 has emerged as a potential player in the complex pathogenesis of HD. The dynamic distribution of δ2R/TMEM97 within neuronal cells and interaction with various proteins, including mHTT, and organelles, are implicated in the pathological cascade of HD. Our *in vitro* HD cell model showed that δ2R/TMEM97 modulators protected neurons from mHTT-induced toxicity with good brain penetration and long half-life in mouse brains. Co-IP experiments indicated that δ2R/TMEM97 interacted with wtHTT/mHTT. Mutant HTT may interfere with δ2R/TMEM97causing its malfunction, resulting in elevated ER stress, abnormal cholesterol homeostasis, lysosomal dysfunction, mHTT clearance impairment, and exacerbating mHTT toxicity. Because manipulating the δ2R/TMEM97 pathway altered mHTT-induced neuronal toxicity, targeting δ2R/TMEM97 pathway is a novel therapeutic strategy for HD treatment.

Poster #42

Jennifer L.H. Johnson, Evan C. Unger

$NanO_2^{TM}$, a novel neuroprotectant for large vein occlusion in acute ischemic stroke

Introduction: Stroke affects more than 795,000 patients per year in the US, kills approximately 140,000 and is the single largest cause of expense for long-term medical care in the US. Costs for stroke are estimated at $34B per year in the US including health care services, medications, and missed days of work. About 87% of strokes are ischemic with large vessel occlusion (LVO) strokes accounting for almost 40% of acute ischemic strokes (AIS) but causing 95% of mortalities and 62% of long-term dependence. NuvOx Pharma is developing a novel oxygen therapeutic, $NanO_2^{TM}$ (2% w/vol dodecafluoropentane emulsion), which if administered early in the care of stroke patients has the potential to keep the at-risk brain tissue alive until reperfusion is attained. $NanO_2^{TM}$ could greatly prolong the window for reperfusion therapies.

Methods: In a Phase Ib/II trial in AIS, patients were treated with three doses of $NanO_2$ ™ or placebo at 0.05, 0.10 or 0.17 mL/kg administered 90 minutes apart. Each dosing cohort consisted of eight subjects randomized 3:1 (6 $NanO_2$ ™, 2 placebo). Oxygenation of brain tissue was demonstrated in a separate Phase Ib/II trial in glioblastoma. TOLD MRI was employed before and after treatment with $NanO_2$ ™ such that the oxygenation of the hypoxic tumor could be seen. In preclinical stroke models, imaging of oxygenated brain tissue with $NanO_2$ ™ was carried out with BOLD MRI and ^{18}F-MISO PET scanning.

Results: In the stroke clinical trial, there were no drug related SAEs and the MTD was not determined. The high dose cohort (0.17 mL/kg) had a significant improvement in the functional end-point of the modified Rankin scale (mRS) at 30 and 90 days. In the forementioned glioblastoma trial, dynamic oxygenation of the brain tumor was visualized. Brain imaging with BOLD MRI and ^{18}F-MISO were also successful in showing the increase in blood oxygenation. The average brain infarct reduction in 8 different animal species was found to be 80%.

Conclusion: $NanO_2$ ™ was found to be both safe and efficacious in AIS animals and patients. It has the potential for administration as a standard of care neuroprotectant. Because low doses of $NanO_2$ ™ are active and the material is safe, it could be deployed early in the care of stroke patients, e.g. in the emergency room or ambulance, to maximize its neuro-protection potential. $NanO_2$ ™ has potential to both save lives and fundamentally improve outcomes in stroke patients. Most recently, the NIHR in the United Kingdom has allowed a prospective clinical trial using $NanO_2$ ™ in treating LVO AIS in 150 patients. NuvOx plans to initiate this trial in early 2024.

Poster #81

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Inhibition of nSMase2 reduces tau transmission in Alzheimer's disease mouse models

Alzheimer's disease (AD) remains the most prevalent form of dementia worldwide, with only minimally effective treatments. AD is clinically characterized by progressive cognitive decline and pathologically by the accumulation of two harmful proteins, AÎ² and pTau. Recent studies have emphasized the role of extracellular vesicle (EV)s in the transmission of pathological tau between cells and have identified the partial inhibition of EV production using small-molecule inhibitors targeting nSMase2 as a potential therapeutic approach. However, no compounds suitable for clinical development have been discovered so far. Using high-throughput screening and extensive medicinal chemistry efforts, we identified PDDC as a highly selective and potent inhibitor of nSMase2. PDDC has excellent brain penetration and is orally bioavailable. To assess the impact of PDDC on tau propagation *in vivo*, we chronically administered PDDC-containing chow to PS19 transgenic mice. Following 5 months of dosing, we evaluated brain ceramide levels, nSMase2 activity, tau levels, glial activation, thickness of the hippocampal neuronal cell layer, and staining of synaptophysin in mossy fibers. Additionally, we isolated and characterized neuronally-derived extracellular vesicles (NDEVs) from plasma. To directly observe the PDDC effect on tau propagation, we also developed a mouse model where a P301L/S320F double mutant human tau-encoding adeno-associated virus (AAV) was stereotaxically injected into the hippocampus, and subsequent transfer to the contralateral dentate gyrus (DG) was monitored. Expression of mutant Tau in neurons lead to elevated nSMase2 activity and increased levels of ceramides *in vitro*. *In vivo*, chronic PDDC treatment restored abnormal levels of multiple ceramide species and nSMase2 enzymatic activity observed in the brains of PS19 mice. PDDC-treated PS19 mice showed reduced levels of total tau and Thr181-pTau in the hippocampus. PDDC treatment also reduced glial activation, increased mossy fiber

synaptophysin immunostaining, and improved thickness of the pyramidal and granule cell layers. Additionally, the concentration of plasma NDEVs was reduced, and their p181-Tau levels were lower in PDDC-treated compared to untreated PS19 mice. A decrease in NDEVs carrying p181-Tau was further confirmed through flow cytometry analysis. In mice treated with PDDC after AAV-hTau seeding, there was a reduction in tau staining intensity in the contralateral dentate gyrus (DG). The data obtained from two AD models using PDDC strongly support the potential use of nSMase2 inhibition as a therapeutic strategy to slow down the spread of tau pathology in AD.